FLOCCULATION AND FLOTATION OF WASTE-GROWN MICROALGAE

By

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TO MY WIFE,
RYANG-KYUN KO LEE

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The research reported here was divided into three main parts. In the first part, the effect of environmental and management variables on biological flocculation of waste-grown microalgae was studied. Divalent cation concentration (Ca^{2+} and Mg^{2+}) was important. An inverse relationship between divalent cation concentration and the degree of bioflocculation was observed. The presence of bacteria had a significant effect on the bioflocculation. Also, good correlation between ash-free biopolymer concentration and bioflocculation was generally observed. However, quality of the biopolymer was critical. When bioflocculation occurred, the extracted biopolymer was of a yellowish, gum-type rather than a whitish, flour-type.

In the second part of this research, use of chitosan was examined as flocculant for algal harvesting. Chitosan flocculation was influenced by pH, degree of turbulence in the

flocculation tank, pretreatment with a strong oxidant, algal species, and the type of biopolymer present. Dose requirements were significantly reduced as bioflocculation increased. This investigation also revealed that dose requirements depended on the quality of exobiopolymer. The whitish, flour-type biopolymer having a high ash content hindered chitosan flocculation, whereas the yellowish, gum-type improved it. Brief mixing of the culture (1-2 days) reduced the dose requirement by up to 40%. This was attributed to reduction in the quantity of whitish, high-ash biopolymer.

In the third part of this research, combined chemical flocculation and autoflotation were examined using pilot and field scale processes with chitosan and alum as flocculants. Positive correlation was observed between dissolved oxygen concentration and rise rate. Rise rate depended entirely on the autoflotation parameters: mean velocity gradient, retention time, and flocculant contact time. Also, rise rate was influenced by the physiological condition of the microalgae and the type of flocculant used. The efficiency of the flocculation-autoflotation process was superior to that of the flocculation-sedimentation process.

CHAPTER 1
DESCRIPTION OF PROBLEM AND RESEARCH GOALS

1.1 Algal biomass production and wastewater treatment: present applications and future trends

For the majority of humanity, now located in the developing countries of the tropics, the most compelling long term problem is the production of food. As the human population increases, the crictical parameter in agriculture must eventually be the yield of edible protein. Ultimately, the most appropriate technology in a given environment will be that which maximizes protein yield with respect to land area, fuel input and, in many cases, water. At the moment, an appropriate means for increasing the human food supply is by the use of microorganisms for such purposes as nitrogen fixation and the production of plant protein. Although heterotrophic bacteria were the first candidates for these roles, some attention has now shifted toward autotrophic microbes, the microalgae, including the cyanobacteria. In a world of diminishing energy supplies, the algae have the desirable attribute of fixing new energy while doing useful metabolic work. Converting the dissolved nutrients in domestic or animal wastes to useful plant protein can be accomplished by algal cultures. This can be done on a continuous basis in tropical or subtropical climates and throughout much of the year in temperate climates. The animal waste generated in the U.S. alone amounts to 30×10^6

kg of manure nitrogen daily (Miner, 1969; Hill, 1974; Calvert, 1974). If this were converted to edible plant protein, it would be sufficient to meet the minimum daily protein requirement for every human on earth (Lincoln, 1981).

The high productivity of microalgae, coupled with the tolerance of many species to high concentrations of nitrogen and phosphorus that are lethal to most aquatic plants, make them especially well suited for on-the-spot recycling of organic wastes. Thus, the culture of microalgae in shallow impoundments has recently received considerable attention as means of treating wastes while producing edible plant protein. By engineering the algal growth units to maximize photosynthesis, the wastewater medium can be rapidly and effectively treated through the uptake of nutrients by the cells and photosynthetic oxygenation. The annual yield of microalgae is potentially in excess of 50 tonnes dry weight/ha in favorable climates (Goldman, 1979). The dry weight yields of algae at least an order of magnitude greater than soybean can be obtained the year round in northern Florida (Lincoln and Hill, 1980). The dried algae typically contain 50% high-grade protein and can be used to replace soybean meal as a animal feed supplement. In spite of the fact that some 90% of the food value is lost in moving to a higher trophic level, the most promising avenue for introducing algae into industrial and developing economies is as an animal feed. Algal meal can be substituted for soybean meal in the diets of poultry (Shelef et al., 1977; Lincoln and Hill, 1980), swine (Hintz and Heitman, 1967; Lee, 1980; Lincoln and Hill, 1980) and ruminants (Hintz et

al., 1966). Furthermore, the filamentous blue-green algae Spirulina have gained considerable attention recently for human consumption as health food. The protein content of this algae is generally in the range of 60 to 65% of the dry weight and the product is somewhat more digestible than green algae. The cost per ton of Spirulina protein is given as $399 versus $794 for soybean protein (Leeslie, 1981). Energy output/input ratio is 4.33 for Spirulina and 1.18 for soybean. By comparison, the cost per ton for pork protein is $20,258 and the energy ratio is 0.17. However, these figures do not take into account the biological feasibility of extensive Spirulina production. Use of Spirulina for large scale recycling of swine wastes is now under investigation in Taiwan.

Besides the recovery of protein from wastes, microalgae have considerable potential for renovation of domestic and animal wastes and for production of fuel and other commercial products. For the majority of such uses, considerable economy can be attained through the use of organic wastes as a nutrient source. A particularly abundant source is the manure produced at a rate of more than 10^8 tonnes (dry weight) annually in the confined livestock operation of the U.S. (Van Dyne and Gilbertson, 1978). Of special importance in this regard is the effluent from anaerobic digesters used for the generation of methane from manure slurries. Algae grown on wastewater effluents can be brought to maturity in dense cultures with little or no chemical supplementation at virtually no cost. In the coming decades, it is probable that methane fermentations on various substrates will

engender widespread development of algal culture technology for the renovation and reclamation of wastewaters and digester effluents.

At present, the major obstacle to the use of algae is the high cost of harvesting the cells which is calculated at 80% of operating expenses. If this cost could be substantially reduced, the production of microalgae from wastes could become profitable.

1.2 Alternative harvesting processes and their rolein algal biomass production system

1.2.1 State of the art in algal harvesting technology

Centrifugation, chemical coagulation/separation, and microstraining have been suggested as methods to remove algal cells which are of extremely small size and are highly dispersed in the growth medium.

Centrifugation operates through the increase of discrete settling velocity by centrifugal force instead of gravitational force. However, this technique requires a large energy input, approximately 2100 kWh of electricity per million litres of effluent (Golueke and Oswald, 1965).

Algal biomass can also be separated by microstraining or ultrafiltration. These methods have limitations, such as loading rate, high maintenance requirements, periodic sand removal or fabric replacement, and high capital cost.

The separation techniques of sedimentation and flotation are commonly used in removing algae from pond effluents. Both processes require chemical flocculants to form flocs initially. Generally, lime, alum, ferric and ferrous salts are used as

chemical coagulants. Long chained polyelectrolytes, which are artificial polymers or natural polymers, are also used for flocculation of dispersed algal cells. Significant concentrations of these chemicals must be used to achieve flocculation in a short period of time. To decrease the chemical dose, chlorination prior to chemical addition was reported to be effective and to enhance the coagulation at natural pH values (Sharma and Venkobachar, 1979). Algal flocs are slow to settle and poorly compacted by gravity, so this process requires low surface loading rates and produces a voluminous sludge. Algal flotation processes enable faster removal and attain denser slurry concentrations than sedimentation. Flotation requires minute gas bubbles which can be variously provided by electrolysis, CO_2 injection, preaeration under pressurized conditions, or high dissolved oxygen (DO) concentration. High concentrations of algae (450 g/m^3 as suspended solids) in swine waste effluent were successfully removed by autoflotation with a high average separation efficiency (Koopman and Lincoln, 1983). However, the chemical coagulation/flotation process requires an input of energy and chemicals, and tends to be excessive in cost. If chemical costs can be reduced or avoided, this process would then be one of the most promising algal harvesting methods. The process could then provide usable amounts of algal biomass with a minimum of space and equipment.

Algal bioflocculation, which is analogous to the bioflocculation observed in activated sludge processes, was observed in isolation ponds and in mechanically mixed high-rate

ponds (Oswald et al., 1978; Eisenberg et al., 1981). Algal bioflocs examined by optical and scanning electron microscopy revealed that the intact algal cells were located in a matrix of zoogleal and filamentous bacteria or bacteria-like microorganisms (Koopman, 1981). The extracellular biopolymers of both algae and bacteria are thought to be important in bioflocculation, which can be partially explained by molecular bridging mechanisms. Algal bioflocculation for the removal of algal cells in pond effluent may be most useful in the reduction or elimination of chemical requirements and for energy conservation. Unfortunately, the performance of algal removal by this process remains inconsistent, and the mechanisms of algal bioflocculation needs further elucidation.

Thus, it is seen that most available technologies for algal separation have problems in terms of cost, energy consumption, complexity and performance. One objective of the present research is to provide information essential to the solution of these problems.

1.2.2 Algal biomass production systems

Ponds may be designed to be deep or shallow with/without mixing. In the former case, thermal stratification (thermocline) exists diurnally and seasonally. Even in shallow oxidation ponds (e.g. high rate pond), thermal stratification and a sharply clinograde dissolved oxygen (DO) distribution are frequently observed (Koopman and Lincoln, 1983). There is generally little mixing of water across an existing thermocline; the epilimnion, the upper zone of water above thermocline, is typically warm and

oxygen rich, while the hypolimnion, the zone of water below thermocline, is generally characterized by lower temperature and low oxygen concentration. The distribution of DO will vary not only with the depth of thermocline, but also with pond dimensions, wind velocity, and the degree of photosynthetic activity. The euphotic zone, which is defined as the depth of light penetration equal to or above the compensation level, is generally rich in dissolved oxygen (DO) during daylight hours as the result of microalgal photosynthesis. Below the compensation level, DO sharply declines to zero. At night, when photosynthetic activity is absent, DO in the eplimnion is taken up by microbial respiration and the concentration eventually approaches zero. The carbon-oxygen cycle of oxidation ponds in shown in Fig. 1-1. Microorganisms, such as bacteria, fungi and yeast, metabolize the organic carbon, producing cell mass and inorganic components: CO_2, CH_4, NH_3, NO_2^-, NO_3^-, PO_4, H_2S, etc. The carbon dioxide is used as a carbon source by algae and cyanobacteria, which grow and produce oxygen as a by-product. Then, the oxygen is used by aerobic bacteria to metabolize the waste organics. The interactions within the microbial community of oxidation ponds are obviously very complex, but there is a predominately mutualistic relationship between the bacteria and the algae. If raw wastewater is introduced to the oxidation ponds, the solids settle to the bottom where they undergo anaerobic decomposition, and the colloidal and dissolved organics are degraded by the bacteria in the liquid. Under anaerobic conditions the solid material is attacked and fermented by nonmethanogenic bacteria, and a portion

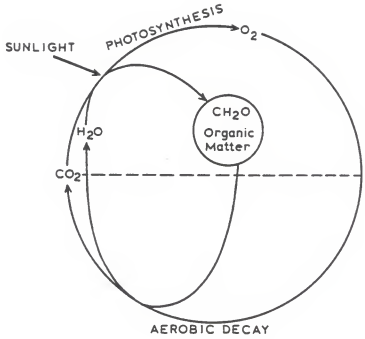


Figure 1-1. Carbon-oxygen cycle of oxidation ponds. (After Gaudy and Gaudy 1980)

of the soluble material is used by the methanogenic bacteria, while the rest is released to the overlying liquid. Soluble, organic end products of anaerobic microbial metabolism may be diffused into upper layers, where they are metabolized under aerobic conditions. Another commomly used oxidation pond system is the high rate pond system, which was developed in the 1960s. This system can be characterized as follows: the oxidation pond is generally shallow (20-90 cm), and the bulk liquid may be completely mixed by a mechanical, paddle-type mixer. Thus, the system can eliminate the thermocline and anaerobic conditions if mixed, so that influent wastewater can be decomposed under aerobic condition during daylight hours. Also, this reduces both the volume of basin and the hydraulic retention time.

1.3 Research goals

The overall goal of this research was to determine how various environmental and operational factors affect the susceptibility of algae produced in mass culture systems to bioflocculation and chemical flocculation by chitosan. Three major research objectives could be identified. The first phase was to gain better understanding of the mechanism of bioflocculation in waste grown algal cultures. The first phase sought to elucidate the contribution of bacteria and their exopolymers to the aggregation process and to devise and test techniques for inducing and promoting algal bioflocculation in laboratory and field scale pond systems. The second phase was to shorten harvesting time and compensate for the inconsistent performance of bioflocculation. Chitosan was introduced for algal

flocculation. The second phase was therefore carried out for evaluating the effects of bioflocculation on algal flocculation with chitosan with emphasis on the reduction of flocculant dose requirements. The third phase was to promote fast separation of algae and obtain well-dewatered product. The third phase focused on algal autoflotation using high concentrations of dissolved oxygen that originate in the water through the photosynthetic activity of algae.

Specific objectives included

1. Test the effect of selected management variables (bifloc seeding, oxidant addition and nutrient composition in medium) on the bioflocculation of algal cultures.

2. Determine the potential for utilization of semi-bioflocculation in combination with chemical flocculation as means of reducing the dosage of chemical flocculant which is required to affect separation.

3. Develop protocol and apparatus for flocculation tests applicable to both field and laboratory algal cultures.

4. Compare available chitosan formulations (e.g., king, snow, and dungeneous crab) in terms of pH optima and flocculation efficiency.

5. Determine the effect of pond operational parameters (e.g., mixing, waste composition and loading, nature and quantity of seeding) on chemical flocculation characteristics of chitosan.

6. Determine the influence of algal population characteristics (e.g., growth phase, algal species, extracellular

polymer composition and concentration) on culture flocculation characteristics.

7. Investigate the relative performance of sedimentation and flotation for separation and thickening of flocculated algae with alum or chitosan.

CHAPTER 2
EXPERIMENTAL APPROACH

2.1 Overview of experiments and research plan

The research was conducted in three phases. In the first phase, the effects of floc forming bacteria, nutrient composition of the basal algal culture medium, type of waste and waste loading rate on bioflocculation in laboratory and field cultures were studied. In the second phase, polyelectrolyte (chitosan) addition to algal culture during the early state of bioflocculation was next tested. Dosage requirements and performance of chitosan flocculation was monitored with algal culture from laboratory scale reactors and field scale ponds. Emphasis was placed on the roles of exopolymer concentration and composition, algal growth phase, major algal species, and pre-mixing conditions. In the third phase, the effects of physicochemical and biological parameters on algal autoflotation with alum and chitosan were tested. Experiments conducted during the three phases of research are summarized in Table 2-1.

2.2 Description of field pond system

Principal components of the field system included raw waste generation, anaerobic/facultative treatment, photosynthetic oxygenation, and algae harvesting. Flushed wastes from pigs housed over concrete slab or slatted floors were regularly

Table 2-1. Summary of experiments

Exp. no.	Dates	Experimental variable	Culture origin/ dominant genera
Phase I: Bioflocculation			
Laboratory experiments:			
L-1	Jan-Feb 85	Nutrient media composition	Lab. grown culture Chlorella/Monodus
L-2	Nov 85	Oxidant addition and bacterial biofloc seeding	C-4 Chlorella/Monodus
Field experiments:			
F-1	Sep-Oct 84	Algal biofloc seeding	C-1 and C-2 Chlorella/Monodus
F-2	Nov 84	Acidification	C-1 and C-2 Chlorella/Monodus
F-3	Mar-Apr 85	Waste loading	C-1 and C-2 Chlorella/Monodus
Phase II: Chitosan flocculation			
Laboratory experiments:			
L-3	Jul-Nov 85	Evaluation of micro-jar test procedure	C-4 Chlorella/Monodus
L-4	Mar 85	pH, chitosan formulation and settling time	C-4 Chlorella/Monodus
L-5	Nov 85	Mixing in the flocculation test	C-4 Chlorella/Monodus
L-6	May 85	Pre-treatment with strong oxidant	C-4 Chlorella/Monodus
L-7	Apr-Sep 85	Algal species	C-4 Chlorella/Monodus Synechocystis
L-8	Jul-Aug 85	Algal growth phase	Laboratory Chlorella/Monodus

Table 2-1 continued

Exp. no.	Dates	Experimental variable	Culture origin/ dominant genera
L-9A L-9B	Sep 85	Mixing in the culture vessel	Laboratory Chlorella/Monodus
L-10 L-11A L-11B	Jan 86	Algal loose polymer	C-4 Chlorella/Monodus
Field experiments:			
F-4	Mar-Apr 85	Waste loading	C-1 and C-2 Chlorella/Monodus
F-5	May-Jun 85	Mixing velocity	C-1 and C-2 Chlorella/Monodus
F-6	Oct 85	Mixing velocity	C-1 and C-2 Synechocystis
F-7	Dec 85	Bacterial biofloc seeding	C-1 and C-2 Chlorella/Monodus
Phase III: Autoflotation Field experiments:			
F-8	Jun-Jul 85	Dissolved oxygen	C-4 Chlorella/Monodus
F-9	Jun-Jul 85	Mean velocity gradient time and retention time	C-4 Chlorella/Monodus
F-10	Jul 85	Flocculant contact time	C-4 Chlorella/Monodus
F-11	Jun-Sep 85	Flocculant dosage	C-4 Chlorella/Monodus Synechocystis
F-12	Jun-Sep 85	Algal species	C-4 Chlorella/Monodus Synechocystis
F-13	Jul 85	Culture characteristics	C-4 Chlorella/Monodus

Table 2-1 continued

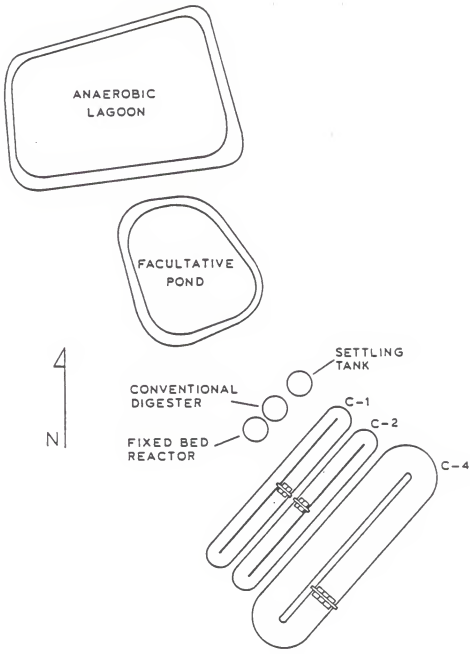
Exp. no.	Dates	Experimental variable	Culture origin/ dominant genera
F-14	Dec 85	Field scale harvesting	C-4 Chlorella/Monodus

Abbreviations:
C-4 = High-rate pond at field microalgae production system
C-1, C-2 = Bioflocculation ponds at field system

discharged to anaerobic treatment (digesters or lagoon) after temporary storage in underground pits. The wastewater discharge rate was variable depending on the number and age of pigs in residence at the barns. Another factor affecting the discharge rate was ambient temperature. Cooling sprays employed during hot weather greatly increased wastewater flows. Based on an average population of 260 animals at mid-cycle and an average wastewater solids concentration of 1%, the estimated flow was 8.7 m^3/d.

The anaerobic digestion system (Fig. 2-1) consisted of a settling tank, conventional digester and fixed bed reactor. A portion (2.8-3.2 m^3/d) of the flushed wastes from the swine confinement buildings was pumped to the settling tank, which was 15 m^3 in volume and constructed of steel. Settled solids from this tank were fed to the conventional anaerobic digester at a rate of 0.9-1.3 m^3/d. The digester was made from a 20 m^3 polyolefin tank and was unmixed and unheated. Its loading rate ranged from 0.14-1.98 kg volatile solids (VS)/m^3-d and averaged 0.67 kg VS/m^3-d. Supernatant from the settling tank (1.9 m^3/d) was fed to the fixed bed reactor, which was operated at ambient temperature. This unit was constructed from a 20 m^3 polyolefin tank and was filled with cypress wood chips which acted as support media for anaerobic bacteria. Its loading rate (empty bed volume basis) ranged from 0.30-1.92 kg VS/m^3-d and averaged 1.08 kg VS/m^3-d.

Excess effluent flows from the digestion system, as well as flushed wastes not pumped to the settling tank, were discharged to the anaerobic lagoon (Fig. 2-1). This impoundment had a depth of



Scale: 1cm = 6.7m

Figure 2-1. Principal components of swine waste treatment process in field system.

4.9 m and surface area of 1 200 m^2. Overflow from the anaerobic lagoon was received by a facultative pond which averaged 1.0 m in depth and had a surface area of 800 m^2. Effluents from the anaerobic digestion system, anaerobic lagoon and facultative pond were used as alternative feed sources for the photosynthetic oxygenation stage (high-rate pond) and algae harvesting stage (bioflocculation ponds).

The high-rate pond (designated C-4) was excavated in heavy clay soil and had a length of 46 m and surface area of 600 m^2 (Fig. 2-1). A racetrack configuration was imparted by a center baffle which extended for most of the pond's length (40 m). Flow-mixing at a velocity of 20 cm/s was achieved by means of an electric motor driven paddlewheel. Mixing was carried out for 30 min three times weekly, coinciding with periods of waste loading. Operating depth of the high-rate pond varied from 0.2-0.4 m. The bioflocculation ponds (designated C-1 and C-2) were identical concrete lined channels, each 38 m long and 170 m^2 in surface area, with center baffles arranged to give a racetrack configuration. Mixing schedules and waste loading were varied according to the experiment in progress. Operating depth ranged from 0.2-0.5 m.

Infestations of zooplankton (e.g., Brachionus rubens, Diaphanosoma brachyurum) were controlled in the high-rate and bioflocculation ponds by addition of concentrated (23% as N) ammonium hydroxide solution (Lincoln et al., 1983). The effect of these additions was to raise the free ammonia concentration. The target free ammonia concentration was 21 g/m^3, a value sufficient

to kill virtually all zooplankton without adversely affecting the algal population. NH_4OH solution was dosed over a period of approximately 10 min while the pond being treated was mixed.

2.3 Analytical methods

2.3.1 Chlorophyll a

Chlorophyll a was determined by a modification of the method of Talling and Driver (1963). A 10 mL of sample was centrifuged for 10 min at 2100 x g and the centrate poured off. A 10 mL of boiling 90% methanol was then added, the tube capped, and the pellet disrupted by vortexing for 15 s. Extraction of chlorophyll a was continued by placing the centrifuge tube in a 70°C water bath for 45 s. Then, the sample was centrifuged at 2100 x g for 10 min and the absorbance of the supernatant measured by absorption spectrophotometry. Chlorophyll a concentration was calculated according the following equation:

$$\text{Chl } a = \frac{13.9\ (D_{665} - D_{750})\ v}{V\ l} \qquad (2\text{-}1)$$

where Chl a has units of g/m^3, D_{665} = optical density at the absorption maximum for Chl a (665 nm), D_{750} = optical density at 750 nm, to correct for turbidity, l = path length (cm), v = volume of extract (mL), and V = volume of sample (mL).

2.3.2 Residue, organic matter and nutrients

Analyses of residue and organic matter were carried out according to APHA (1980). These included total solids (209A), volatile solids (209E), total suspended solids (209D), volatile suspended solids (209G), and chemical oxygen demand (508A).

Filtrations were on Whatman GF/C glass fibre filters which have an average pore opening of 1.2 μm. Ammonia and total Kjeldahl nitrogen were determined according to the distillation (00610) and digestion (00625) procedures described in EPA (1974). Total phosphorus was determined by the persulfate digestion procedure (00665) of EPA (1974).

2.3.3 Visibility and estimation of volatile suspended solids in field experiments

Visibility was measured using a 15-cm diameter secchi disc. The depth at which the disc disappeared from sight as it was lowered in the culture was taken as the visibility.

Volatile suspended solids concentration was estimated according to following equation:

$$VSS\ (g/m^3) = 750/d \qquad (2\text{-}2)$$

where d = is the extinction distance in inches of the submerged Secchi disk. This method was empirically developed in ponds of the University of Florida by Lincoln and Hill (1980). They showed that with this method, the combined algal and bacterial density was measured as VSS rather than algae alone.

2.3.4 Algae removal efficiency

The algae removal efficiency was calculated on the basis of either Chl. *a* or optical density. The term "chlorophyll removal efficiency" is applied to removal percentages based on chlorophyll measurements, whereas the term "algae removal efficiency" is applied to removal efficiency based on optical density measurements.

2.3.5 Settleable matter and settling efficiency

Settleability of algae in the field experiments was determined by retaining a sample of culture medium in a 1 litre Imhoff cone over a 24 h period in the absence of light. Accumulation of algae on the sides of the cone was minimized by gently swabbing the inside of the cone with a rod after an initial settling period of 2 h. Volume of settled matter at the bottom of the cone was measured after an additional 22 h of retention. Decrease of algal biomass in the medium was calculated by comparing the initial Chl a concentration of the sample to the Chl a concentration of a 150 mL supernatant sample taken at the end of the 24 h settling period. In laboratory experiments, settling tests were conducted in a 100 mL graduate cylinder for 1 hour in the absence of light. Removal efficiencies were calculated by comparing the optical density of a 10 mL supernatant sample to the optical density of the original sample.

2.3.6 Dehydrogenase activity

Dehydrogenase activity was measured by a modification of the technique of Koopman et al. (1984). Sample pH was adjusted to 8.7 with 0.1 N sodium hydroxide. Triplicate 10 mL aliquots were amended with 1.0 mL 0.2% 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyltetrazolium chloride (INT) (Eastman Kodak) and incubated at room temperature (22 $\pm$ 2°C) in the dark. Incubation was terminated by adding 1 mL 37% formaldehyde. INT-formazan (INTF) formed during the incubation period was extracted according to the following procedure. A 10 mL INT-treated sample was placed in a centrifuge tube and centrifuged at 2100 x g for 20 min. Centrate

was decanted, leaving a pellet approximately 0.1 mL in volume, and replaced with 10 mL 4+6 tetrachloroethylene/acetone. The tube was capped, then vortexed for 15 s. Extraction was continued in the dark for 30 min. Extract was clarified by centrifugation at 2100 x g for 20 min and optical density of the extract determined at 490 nm. INT-dehydrogenase activity (INT-DHA) was calculated according to the following equation (Lopez et al. 1986):

$$\text{INT-DHA} = \frac{1024\ D_{490}\ v}{V\ t\ F} \qquad (2\text{-}3)$$

where INT-DHA is expressed in units of equivalent oxygen uptake (g O_2*/m^3 d), v = volume of extract (mL), V = volume of sample (mL), t = incubation time (min), and F = factor to account for sample dilution by INT and formaldehyde (0.833).

2.3.7 Biopolymer concentration

Exopolymers were determined according to the technique of Ueda (1963). A 45 mL sample was shaken vigorously for 20 s and centrifuged at 10,000 x g for 20 min at 4° C. A 30 mL centrate was combined with 60 mL ethanol in a vial and stored at 4° C for 24-48 h. The resulting precipitate (whitish or yellowish material) was separated by centrifugation at 10,000 x g for 10 min at 4° C. Following centrifugation, the centrate was discarded and the pellet transferred with several washings of distilled water to a pre-weighed crucible. Additional weighings were taken after 24 h drying at 103° C and 15 min ashing at 550° C. Biopolymer was expressed in terms of total dry weight or ash-free dry weight per unit volume (g/m^3) or per unit mass of suspended solids (g/g TSS).

2.3.8 Biopolymer composition

Simple sugars, oligosaccharides, polysaccharides, and their derivatives were measured by the phenol-sulfuric acid method of Dubois et al. (1956). In this assay, 10 mL of the biopolymer/ethanol mixture were centrifuged at 2100 x g for 15 min. The pellet was redissolved with 5 mL of distilled water. A 2 mL of this solution was pipetted into a 10 mL glass test tube and 1 mL 5% phenol (95 mL of distilled water + 5 grams of redistilled reagent grade phenol) was added. Then, 5 mL of concentrated sulfuric acid (reagent grade 95.5 % conforming to ACS specifications) was added rapidly, the stream of acid being directed against the liquid surface rather than against the side of the test tube in order to obtain good mixing. The tubes were allowed to stand 10 min., then they were shaken and placed for 15 min in a water bath at 27° C. The absorbance of the characteristic yellow orange color was measured at 490 nm for glucose. Blanks were prepared by substituting distilled water for the sugar solution.

Protein was determined by the modified Lowry phenol method of Oyama and Eagle (1956) as detailed in Pelczar and Chan (1977). In this assay, 45 mL of the biopolymer/ethanol mixture was centrifuged at 5000 x g for 10 min at 4° C. The pellet was dried in a desicator under vaccum for 3 hours. A 6 mL of N/10 NaOH was the pipetted into the centrifuge tube. The tube was capped, then vortexed for 30 s. The sample was then allowed to stand at room temperature for 1 day to obtain complete dissolution.

Reagent A was prepared by dissolving 20 g of Na_2CO_3 and 0.2 g of NaK tartrate in 1000 mL of distilled water. Reagent B was prepared by dissolving 5.0 g of $CuSO_4$ $5H_2O$ in 1000 mL of distilled water. Reagent C was made from combining 50 parts of reagent A with 1 part of reagent B before use. Folin-Ciocalteau reagent was prepared by the dilution of 5 mL of reagent grade Folin-Ciocalteau solution (2 N, Fisher) with 7 mL of distilled water. A 1 mL of sample was added to 4 mL of reagent C and then mixed by hand shaking. The sample was allowed to stand at room temperature for 10 min; 0.5 mL of Folin-Ciocalteau reagent was then added and color development was allowed to occur at room temperature over a period of 30 min. Absorbance was read at 690 nm. A standard curve was prepared using bovine serum albumin fraction V as the protein standard.

Nucleic acid was estimated according to Warburg and Christian (1941). A 5 mL of N/10 NaOH and polymer mixture prepared according to procedure described in section 2.3.8 was centrifuged at 2400 x g for 20 mim. A 2 mL of supernatant was pipetted into a vial. Absorbance at 280 nm and 260 nm was read through a uv spectrophotometer using a 1 cm quartz cuvette. Nucleic acid concentration was calculated based on the ratio of absorbance at 280 nm to absorbance at 260 nm and the protein concentration. The correlation between the ratio of optical densities and the nucleic acid content of protein as reported by Warburg and Christian (1941) is given in Figure 2-2.

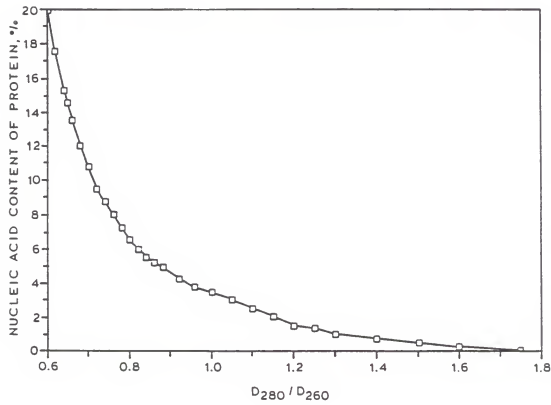


Figure 2-2. Relationship between the nucleic acid content of protein and the ratio of optical densities at 280 nm and 260 nm.

2.3.9 pH and dissolved oxygen

pH measurements were made with an Orion Model 601A electrode/analyzer system. Dissolved oxygen (DO) was determined with a YSI Model 54A oxygen meter and polarographic electrode. The DO and pH of the bioflocculation ponds were measured at a depth of 0.05 m between 2 pm and 5 pm local time.

PHASE I.

BIOFLOCCULATION

CHAPTER 3
EFFECT OF ENVIRONMENTAL AND MANAGEMENT VARIABLES ON BIOLOGICAL FLOCCULATION OF WASTE-GROWN MICROALGAE

3.1 Introduction

The purpose of phase I of this study was to determine the effects of the nutrient composition of algal basal medium, waste type and waste loading rate, biofloc bacterial seeding, culture mixing, oxidant addition, and extracellular polymer concentration on bioflocculation of waste grown microalgae.

3.2 Literature review

3.2.1 Polyelectrolytes and bridging theory

Early researchers attempted to explain the aggregation of microorganisms on the basis of purely physicochemical considerations, emphasizing the summation of physical forces, such as van der Waals forces, electrical repulsion, etc. These considerations alone cannot explain the behavior of microbial suspensions. For example, the stability of certain solids near their isoelectric point and aggregation of negatively charged colloids with nonionic or anionic polymers (Northrop and Dekruif, 1921-1922; Busch and Stumm, 1968) cannot be accounted for by this theory.

It is plausible to interpret microbial aggregation in terms of polyelectrolyte interactions (Tenney and Stumm, 1965; Pavoni et al., 1972; Harris and Mitchell, 1973; Dugan et al., 1970).

Natural polymers, such as complex polysaccharides, polyaminoacids, and polynucleotides, are excreted or exposed at the surface of microorganisms. These high-molecular-weight extracellular biopolymers, which have accumulated at the microbial surface and extended into the water phase, are one of the main factors in the bioflocculation of dispersed cells. Extracellular biopolymers bond to cells electrostatically or physicochemically and subsequently bridge between cells to form a three dimensional matrix.

Polyelectrolytes are linear or branched chains of small subunits, which contain ionizable groups (e.g., -COOH, -OH, $-NH_2^+$, $-R_1NR_2^+$). They are soluble in water and are affected by electrostatic forces between their charged sites. Natural polyelectrolytes include proteins, nucleic, pectic, and alginic acids, polysaccharides, and numerous polyacids. Synthetic polyelectrolytes are formed by polymerization of simple monomers. Polyelectrolytes can be classified as cationic, anionic, or amphoteric. Nonionic polymers may also be classed as polyelectrolytes if their neutral charge is due to equal summations of positive and negative charges. Aggregation of colloidal microorganisms with polymers can be achieved by adsorption of the polymer onto the solid, cross-linkage of the segments of the polymer to form bridges between the dispersed microorganisms, and formation of a loose, three-dimensional structure. The interaction of adsorbed and extended lengths of polyelectrolytes with the vacant bonding sites of adjacent microrganisms can form bridges and can induce eventual growth of

the aggregate (LaMer and Healy, 1963; Harris and Mitchell, 1973). The bridging model is shown schematically in Figure 3-1.

The following physicochemical variables are important in ploymer-induced coagulation:

(a) Polymer concentration--Bridging between particles is optimal when the particles are half-covered with adsorbed segments of the polymer. More extensive coverage leads to redispersion of the suspension.

(b) Intensity and time of agitation--Optimal time and intensity of agitation exist for maximum aggregation. Excessive mixing intensity can decrease the amount of polymer adsorbed, whereas extended agitation periods can decrease the degree of aggregation by reducing the extended length and number of adsorbed polymers.

(c) Qualitative properties of polymer--Elongation of the polymer chain enhances the probability of bridge formation. For significant aggregation of colloidal particles with nonionic polymer or polymers carrying the same charge as the particles, polymer molecular weights in excess of several hundred thousand are required, whereas the counter ionic charged polymers are effective at lower molecular weights.

(d) pH in solution--The pH in solution has a pronounced effect on polymer-induced aggregation by its influence on electrical double layer thickness and on elongation and ionization of polymers.

(e) Electrolyte concentration--Polyvalent electrolytes are particularly significant to the aggregation of nonionic or like

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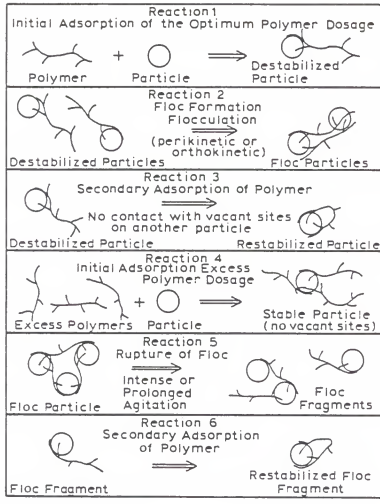

Figure 3-1. Schematic representation of the bridging model for the destabilization of algal cells by polymers. (After O'Melia 1972)

charged polymers and particles. A significant increase in ionic strength will normally decrease the extent of polymer adsorption on solids owing to competition for adsorption sites by inorganic ions that are charged similarly to the polymer.

3.2.2 Extracellular polymer production

Extracellular biopolymers of microorganisms originate from cell lysis, excretion, and extracellular synthesis. The amount of extracellular polymer produced is related to the growth phase of the microorganisms. Several investigators have observed that production of extracellular polymers, notably polysaccharides, by unicellular algae significantly increases during the late logarithmic phase of growth and continues until slightly after cells reach the stationary phase (Lewin, 1956; Moore and Tischer, 1964). Pavoni et al. (1972) reported that the total amount of biopolymers accumulated in the bacterial culture increased sharply after the bacteria entered the endogenous growth phase. This behavior was most pronounced under carbon limiting conditions. Pavoni et al. (1972) suggested that the majority of biopolymer is from autolysis. This would explain why large quantities of protein, RNA and DNA were detected in microbial polymers extracted during the later stages of growth (Dunican and Seeley, 1965; Pavoni et al., 1972; Sakka et al., 1981). Conversely, Gulas et al. (1979) observed that the exopolymer content per unit biomass decreased at increasing sludge ages in continuous activated sludge reactors. They concluded that the maximum activity of autolytic enzymes occurs during the early phase of logarithmic growth, and, as sludge age increases, autolysis of cells slows. A similar

result was reported by Rudd et al. (1984), who found that the total polysaccharide in activated sludge per gram SS varied in proportion to the food-to-microorganism ratio and inversely with sludge age. The concentration of soluble polymer decreased at higher sludge ages.

Extracellular polysaccharides are believed to be synthesized from monosaccharides and simple substrates through phosphate ester intermediates. Enzymes involved in exopolysaccharide synthesis are associated with the membrane underlying the cell wall and are usually observed to be particulate complexes. Lipoidal transport components, notably polyphenols, may transport polar monosaccharide and oligosaccharide repeating unit precursors through the nonpolar cell membranes to the exterior where polymerization can occur (Slodki and Cadmus, 1978). In Streptococcus sp., three enzymes were reported to be involved in the production of glucan exopolymer from sucrose: invertase, glucocyltransferase, and fructosyltransferase (Costerton et al., 1978). Dunican and Seeley (1965) attributed the variability of glucan synthesis by Lactobacillus sp. to enzyme deficiencies of spontanously occurring mutants.

When growth is limited by certain nutrients (e.g., nitrogen, phosphorus, sulfur) in the presence of excess carbon, microorganisms generally produce less protein, more lipids and more polysaccharides than when the same cells are grown in carbon limited media. Duguid and Wilkinson (1953) observed that with decreasing concentration of the nitrogen, sulfur, or phosphorus source until it became limiting, the amount of polysaccharide

produced per cell of Klebsiella aerogenes rose to a maximal level. The maximal polysaccharide:nitrogen ratio of cells was 32 for nitrogen deficient, 40 for phosphorus deficient, and 17 for sulfur deficient cultures. The rate of polysaccharide synthesis was highest during the logarithmic growth phase and diminished progressively thereafter. In batch cultures of Aureobasidium pullulans, polysaccharide production commenced on reaching nitrogen limiting conditions. The presence of free ammonium ion in the medium appeared to supress polysaccharide elaboration (Seviour and Kristiansen, 1983). A similar result was observed in the activated sludge process, where sludge adapted to low ammonium concentration produced higher amounts of extractable polysaccharides (Salanitro et al., 1983). Rudd et al. (1984) suggested that a higher C:N ratio could enhance the production of capsular rather than soluble material. This could result in a more dispersed floc formation and higher SVI value.

Gauthier (1981) reported that oxygen concentration was significant to formation of flocs. Under oxygen limiting conditions, a higher percentage of dispersed growth was observed in continuous cultures of Zoogloea ramigera. A similar result was observed at full-scale plants operated with low DO concentrations in the aeration basins (Starkey and Karr, 1984). At low DO concentration, exocellular polymer production was inhibited, resulting in increased effluent turbidity.

The presence of lytic products of other microorganisms may promote bioflocculation by stimulating growth of zoogloeal bacteria. The floc forming bacteria, Z. ramigera and

Z. filipendula, did not utilize carbohydrate compounds (Dugan and Lundgren, 1960; Tezuka, 1973). Low molecular weight carbon sources (e.g., alcohol, acetate, and glycerol) were metabolized by zoogloeal strains which led to small growth increases with acetate and glycerol and a sizable increase with ethanol. Growth of Zopgloea sp. was greatly stimulated by nucleotides and combinations of purine and pyrimidine, or biotine, folic acid and vitamin B12 (Dugan and Lundgren, 1960). Zoogloea sp. preferentially utilized amino acids as a carbon and energy source when grown in the presence of glucose and amino acids (Gauthier, 1981). Zoogloeal strains were reported to have varying abilities to produce the enzymes necessary for degradation of polymeric substrates, whereas non-floc forming bacteria, such as Cytophaga strains, grew very well in carbohydrate medium. The Cytophaga strains produced gelatinase and dextranase, whereas Zoogloea strains produced no gelatinase and only small amounts of dextranase. Zoogloea strains grew very well with Cytophaga strains in carbohydrate medium (Gude, 1982). Gauthier (1981) observed that even Zoogloea strains lost their floc forming trait in pure culture. He suggested that the study of floc formation should be conducted with mixed populations, under conditions where there is a selective pressure to maintain floc forming ability.

Extracellular polymer synthesis was observed by Parsons and Dugan (1971) to increase as a function of the concentration of carbon source in the medium. The accumulation of poly-beta-hydroxybutyrate (PHB) in the capsule of Zoogloea was observed to follow the rapid initial uptake of carbohydrate

(Crabtree et al., 1965; Parsons and Dugan, 1971). The supernatant viscosity of whole cultures increased as PHB in the cells decreased.

3.2.3 Characteristics of extracellular polymers

Biopolymers can range from simple polysaccharides assembled from identical monomeric units to complex muco-lipo-proteinaceous polymers and protein-nucleic acid polymers of nuclear origin (Harris and Mitchell, 1973). The chemical composition of extracted biopolymer depends on the types of microbial species present and their carbon sources. The composition of biopolymers extracted from activated sludge was H, 5%; C, 35 to 36%; N, 5 to 7%; ash, 13 to 17% (Sato and Osse, 1980). The extracted material contained protein, carbohydrate, RNA, and DNA. Relative proportions of these molecules varied considerably with the extraction method and biomass charateristics (Sato and Osse, 1980; Brown and Lester, 1980). Carbohydrates contained pentose, hexoses, and methylpentoses. Specific compounds detected included arabinose, manose, galactose, glucose, and fructose, as well as others (Table 3-1). Small amounts of aminosugar (glucosamine or galactosamine), proteins and nucleic acids were observed in the extracellular biopolymers of activated sludge (Sato and Osse, 1980). The composition of algal secretions consisted of complex carbohydrates, organic acids and nitrogenous materials (i.e., polypeptides and free amino acids). The monosaccharide components of the extracellular polysaccharides of algae included hexose, uronic acid, pentose and methylpentose. The most common specific

Table 3-1. Extracellular carbohydrates detected in activated sludge.

Reference	Glu	Gal	Man	Fuc	Rhm	Xyl	Ara	Glt	Glm	Gluc	Fruc
Coakley, 1969	+	-	+	-	+	+		-			
Takiguchi, 1972	+		+	+	+		+	+			
Wallen and Davis, 1972	+	+	+		+	+			+		
Forster, 1975	-	+	+	+	-	-		+	-		
Rideau and Marfaux, 1976	+	+			+	+	+			+	+
Steiner et al., 1976	+	+	+	+	+	+		+	-		
Sato and Ose, 1980	+	+	+				+		+		
Mikell, 1981	+	+	+		+	+		+	+	+	+

Glu = Glucose
Gal = Galactose
Man = Mannose
Fuc = Fucose
Xyl = Xylose
Ara = Arabinose
Glt = Glucuronolactone
Glm = Glucosamine
Gluc = Glucuronate
Rham = Rhamnose
Fruc = Fructose
+ = detected
- = undetected

compounds were glucose, arabinose, fucose, and glucuronic acid (Table 3-2).

Floc forming bacteria, such as Zoogloea ramigera and Klebsiella (Aerobacter) aerogenes, can produce vast amounts of extracellular biopolymer, up to 40 and 50 percent of cell weight, respectively, in pure culture (Parsons and Dugan, 1971; Rudd et al., 1983). The major components of this exopolymer are carbohydrate and hexuronic acid. Obayashi and Gaudy (1973) observed that the content of carbohydrate and hexuronic acid of exobiopolymer was 71 and 27.2 percent, respectively, for K. aerogenes and 85 and 0 percent, respectively, for Z. ramigera. Hexose sugar and hexuronic acid concentrations have been used as measures of extracellular biopolymer, including capsular or slime materials (Obayashi and Gaudy, 1973; Brown and Lester, 1980; Rudd et al., 1983), whereas protein and DNA concentrations have been used as measures of the degree of cellular disruption (Brown and Lester, 1980; Rudd et al.,1983).

The distinction between extracellular and intracellular materials of activated sludge may be less distinct than in pure cultures, because vast amounts of protein and nucleic acids are released by the lysis of dead microorganisms. Rudd et al. (1983) postulated that the total amount of extracellular carbohydrate would be 6% of cell weight in activated sludge. They found that extracellular polymers of activated sludge contained protein and carbohydrate in a ratio of approximately 3:1, which was equal to the overall protein to carbohydrate ratio of intracellular matter (Brown and Lester, 1980; Rudd et al., 1983). The overall protein-

Table 3-2. Monosaccharides detected in extracellular material extracted from algal suspensions. After Moore and Tischer (1964)

Algal species	Glu	Gal	Ara	Xyl	Rib	Gluc	Fuc	Rhm
Chlamydomonas Sp.	+			+			+	
Nostoc Sp.	+		+			+	+	
Chlorella ellipsoidea	+		+			+	+	
Chlorella vulgaris	+			+		+		+
Palmella mucosa	+		+			+	+	
Oocystis Sp.		+	+			+	+	
Chlorella Sp.	+		+			+		+
Anabaena flos-aquae	+			+	+	+		

Glu = Glucose Gal = Galactose Ara = Arabinose Xyl = Xylose
Rib = Ribose Fuc = Fucose Rhm = Rhamnose
Gluc = Glucuronic acid
+ = detected
- = undetected

carbohydrate ratio for activated sludge was reported to be a function of F/M ratio and nitrogen concentration (Wu et al., 1982). They found that, in activated sludge processes operated at high F/M ratio under nitrogen deficient conditions, the carbohydrate content of extracellular polymer was increased. In pure cultures of exopolymer producing bacteria, such as K. aerogenes, Z. ramigera, Arthrobacter viscosus and Xanthomonas campestris, the content of protein of exopolymer was 1, 1.4, 10, and 8.1 percent, respectively (Obayashi and Gaudy, 1973).

The biodegradability of the extracellular biopolymers of activated sludge is very low, as indicated by a BOD_5:COD ratio of 0.1 (Pavoni et al., 1972). Therefore, extracellular biopolymer surrounding and bonding to the micobial surface during endogenous growth stages will not be assimilated to any great extent by the microorganisms as an organic substrate (Pavoni et al., 1972).

3.2.4 Measurement of extracellular polymers

The forms of extracellular polymer produced may be classified as loose slime, capsule and microcapsule. Slime polymers remain in the dissolved form and increase the viscosity of surrounding medium, whereas capsular and microcapsular extracellular polymers, which cover the cell wall, are attached to the microorganism and can enhance the settleability of cells by promoting floc formation. Wilkinson (1958) defined arbitrarily that the capsule is visible by light microscopy and its thickness is greater than 200 nm, whereas the microcapsule cannot be visualized with the light microscope and is under 200 nm in thickness.

Some strains of bacteria (e.g. Aerobacter cloacae) produce a structure of extracellular polymer intermediate between a capsule and slime (Wilkinson et al., 1954). The genus Zoogloea produces a gelatineous matrix in the form of packets surrounding cells, which are analogous to capsules (Friedman and Dugan, 1968). Also, cells producing capsules were reported to produce slime material which had a composition similar to capsular material (Wilkinson, 1958).

A variety of extraction techniques have been developed (Table 3-3). These may be classified into two main groups, physical and chemical techniques. The former includes high speed centrifugation, sonication, shear press and homogenization, while the latter includes hydrolysis by acid, alkali or heat, and extraction by organic solvents. Extracted biopolymers are generally harvested by chemical precipitation and centrifugation. Methanol, ethanol or acetone is used for purification of extracted biopolymer. Novak and Haugen (1981) found that the concentration of biopolymer achieved with acetone precipitation was more than that with ethanol precipitation.

Centrifugation methods have commonly been used to strip out extracellular biopolymer by shear forces generated during high speed centrifugation (Lewin, 1956; Wilkinson, 1958; Pavoni et al., 1972; 1974; Kiff and Thompson, 1979; Brown and Lester, 1980; Novak and Haugen, 1981; Gehr and Henry, 1983). This approach was recommended as the most reliable technique for extracting the exobiopolymer of free bacterial cells in suspension (Brown and Lester, 1980). However, this approach was not able to extract significantly the exobiopolymer of activated sludge because the

Table 3-3. Exobiopolymer yields from activated sludge reported for different extraction techniques

Extraction method	Modification	Yield, % TSS	Reference
Centrifugation	Sharples high-speed centrifuge	5.8	Wase & Balasundaram (1980)
Centrifugation	15 min at 36 500 x g	2.6-5.4	Kiff & Thompson (1979)
Centrifugation	15 min at 13 200 x g	2.17-4.17[α] 10.97-18.01[β]	Gehr and Henry (1983)
Boiling	successive 10-min extractions	1.70 ± 1.05[γ] 4.25 ± 0.31[δ]	WPRL (1971)
Heat	1 hour at 80°C, centrifuged 15 min at 10 000 x g	3.4-3.6	Kiff & Thompson (1979)
Heat	1 hour at 80°C	1.13-6.47	Kiff (1978)
Heat	1 hour at 100°C	4.6 (0.9-9.1)	Clarke and Forster (1982)
Steaming	ultrasonication pretreatment, 10 min steaming in autoclave	4.7 (4.2-5.2)	Brown & Lester (1982)
Alkaline extraction	0.1 N NaOH 2.0 N NaOH	1.12 4.55	Sato and Ose (1975)
Boiling and alkaline extraction	ethanol precipitation	2.7	Wallen & Davis (1972)

[α] sludge samples from plant treating domestic wastewater
[β] sludge samples from pilot plant treating synthetic wastewater with glucose as primary energy source
[γ] samples from "conventional activated sludge treatment plant"
[δ] samples from three different activated sludge plants

vast majority of flocs settled before maximal shear force was attained (Brown and Lester, 1980; Novak and Haugen, 1981).

Heat extraction, including steaming and boiling techniques, has also been widely used for the extraction of microbial biopolymer (Wallen and Davis, 1972; Kiff and Thompson, 1979; Brown and Lester, 1980; Clarke and Foster, 1982; Rudd et al., 1983). The main mechanism involved in this method is the hydrolysis of exobiopolymers by elevated temperature. The steaming technique was developed as an alternative to boiling because of the destructive effect of boiling on cells and was recommended by Brown and Lester (1980) for the extraction of the exopolymer of activated sludge. The boiling technique was also reported to give a reproducible, quantitative extraction of the exopolymer of activated sludge (Clarke and Foster, 1982). The amount of exopolymer extracted by this technique was shown to have a significant relationship to the settling properties of the sludge (Clarke and Foster, 1982). Kiff and Thompson (1979) reported that the polymer obtained by this method contained significant amounts of intracellular materials and was denatured. Rudd et al. (1983) demonstrated the effectiveness of various extraction methods by comparing the released carbohydrate concentration with the estimated total carbohydrate present in the exopolymer and also by comparing the ratio of protein to carbohydrate in extracted exopolymer. They suggested that the estimation of cellular destruction by heat treatment might be slightly inaccurate, because a significant reduction of protein concentration of exopolymer was observed while similar amounts of carbohydrate were

found in steam-extracted exopolymer and untreated samples. On this basis, they concluded that the steaming technique was more reliable than the boiling technique.

The solubility of fibrillar biopolymers is increased in alkaline solution. Thus, many researchers have used sodium hydroxide solution for the hydrolysis of exobiopolymers (Takiguchi, 1968; Sato and Ose, 1980; Brown and Lester, 1980; Rudd et al., 1983). Although sodium hydroxide treatments released significant quantities of hexose sugar from activated sludge, this method was thought to cause a vast amount of cellular disruption (Farrah and Unz, 1976; Brown and Lester, 1980). Also, Sato and Ose (1980) demonstrated that the chemical composition of extracted exobiopolymers varied in relation to the applied concentration of sodium hydroxide. The average relative standard deviation of this method was similar to that of the steaming and EDTA treatment methods for biochemical tests in activated sludge, synthetic activated sludge and K. aerogenes culture (Brown and Lester, 1980). Rudd et al. (1983) suggested that rapid hydrolysis methods, such as alkaline treatment or heat treatment, might be useful for comparing the relative production of carbohydrate in different sludges.

Ethylene diamine tetraacetate (EDTA) solution was successfully used to deflocculate yeast (Stahl et al., 1983) and Flavobacterium sp. (Endo et al., 1976; Endo and Takahashi, 1980; 1981). Divalent cations (e.g. Ca^{++}), which are an important factor in the flocculation of biomass, can be removed by chelating agents, such as EDTA. Brown and Lester (1980) showed that

extracted biomass was severely destroyed so that a vast amount of intracellular biopolymer could be extracted by this method.

Physical turbulence generated by ultrasonication or a homogenizer has been used as a method to destabilize aggregated microorganisms prior to enumeration of activated sludge bacteria (Pike et al., 1972). Kiff and Thompson (1979) suggested that sonication was a very effective method of deflocculation and recommended that a combination of low level sonication with high speed centrifugation would possibly be the ideal method for extracting exobiopolymers. Brown and Lester (1980) observed that only a part of the total exopolymer was extracted by the combined method. They suggested that this method would be useful as a preliminary treatment because it avoided significant cellular disruption.

A homogenization technique was used to strip out exopolymer. This method would probably cause the least damage to micoorganisms, but the yield of extracted biopolymer was low (Kiff and Thompson, 1979; Rudd et al., 1983).

Kiff and Thompson (1979) obtained significant amounts of extracellular biopolymer by a "bel" cream-maker, with which the sample was extruded two times under pressure through a narrow orifice. They reported that polymer yield by this shear press method was comparable to heat extraction and gave reasonable reproducibility.

Novak and Haugen (1981) found that qualitatively different biopolymers could be obtained by repeated elution of activated sludge with distilled water which removed divalent cations, but

did not consider this to be a quantitative extraction method. Rudd et al. (1983) examined ion-exchange with Dowex resin as a method to obtain exobiopolymers quantitatively. The principle of this technique is that divalent cations are exchanged for sodium ions of Dowex chemical (Dowex 50-X8, Na^+ form). This chemical does not cause denaturation of proteins by heat or excess pH, removes the various divalent cations, and reduces excessively disruptive effects. They observed that the addition of Dowex chemical to any extraction method could increase the polymer yield.

In conclusion, even though various extraction techniques have been used, none of the above methods can be considered to be quantitative (Novak and Haugen, 1981; Rudd et al., 1983). Rudd et al. (1983) recommended that the rapid hydrolysis method (e.g. heat treatment or alkaline extraction method) might be useful for conglomerates of whole viable, dead and disintegrating cells, bound together by a gell matrix composed of extracellular metabolites and intracellular products of lysis.

3.2.5 Bacterial bioflocculation

Aggregation of bacteria with extracellular biopolymers as well as synthetic organic polymers can be interpreted in terms of a bridging model which involves sorptive interactions between the extracellular biopolymers and active sites of the bacterial surface. Macromolecules such as complex polysaccharides, proteins and nucleic acids are accumulated at the bacterial surface under varying physiological and nutritional conditions as described before. They can be extended, hydrated and ionized so that they

act as polyelectrolytes to form bridges between bacteria from polymeric interactions.

Exopolymers of floc forming bacteria can differ both structurally and chemically. Characteristics of exopolymers from even a single species can differ with carbon source, nutrients and environmental conditions (Costerton et al., 1978). The more soluble extracellular biopolymers are easily sloughed off cells, increasing the viscosity of surrounding medium. The synthesis of relatively insoluble extracellular biopolymers, which can entangle cells and other suspended solids, seems to be the key feature in bioflocculation. The newly synthesized extracellular biopolymers in the logarithmic growth phase can be slowly modified to be more insoluble forms. This might explain why bioflocculation is usually incomplete at the maximal growth phase (Dugan and Pikrum, 1972). Gulas et al. (1979) reported that even though large quantities of extracellular biopolymer were detected at the lower cell ages, pinpoint flocs were observed. These investigators concluded that exopolymers released by autolysis of cells in the logarithmic growth phase consisted of low molecular weight molecules which had little ability to promote aggregation of microorganisms.

Zoogloea ramigera, which may be an important species in the activated sludge process, produces a gelatinous matrix that containing only glucose, that resembles cellulose, having beta-1, 4 linkages. This material entrapped other cells and particulate materials, forming flocs (Friedman et al., 1968; 1969). Tezuka (1973) reported that extracellular biopolymers produced by the

genus Zoogloea were quite different in their chemical composition from those described by Friedman et al. (1968; 1969), which were mucopolysaccharides composed of two aminosugars (N-acetylglucosamine and N-acetylfucosamine).

Table 3-4 shows the binding materials (cellulose, mucopolysaccharide, proteins, nucleic acids) causing the aggregation of various microorganisms. These binding materials were identified with enzyme treatments which completely destroyed the aggregates and appeared to remove much of adhesive material surrounding cells (Harris and Mitchell, 1973).

Endo et al. (1976; 1980; 1981) found the binding material of Flavobacterium included protein as well as divalent cations which can form ionic bonds with negatively charged exobiopolymers. They showed that extracted cells from various growth phases were reconstituted with the purified binding material which represented only a certain portion of the extracted proteins (the so called, aggregation factor) and was thought to be under genetic control.

Mucopolysaccharide was thought to be the main binding material causing the aggregation of Pseudomonas which was isolated from phenol-adapted activated sludge. However, this material constituted only 10% of the total polysaccharides from Pseudomonas, whereas the other 90% of the polysaccharides could not be shown to contribute to floc formation (Tago and Aida, 1975; 1977).

The relatively small amount of double stranded DNA was one of the factors involved in floc formation by Pseudomonas isolated from glycerol-adapted activated sludge. Denatured DNA and DNA

Table 3-4. Binding materials of flocculated microorganisms

Species	Binding material	Deflocculation enzyme	Reference
Corynebacterium xerosis	protein	papain (proteolytic enzyme)	Stanley & Rose (1967)
Pseudomonas aeruginosa	polysaccharide	hyaluronidase	Warren & Gray (1955)
Acetobacter xylinium, Rhizobium sp., Agrobacterium sp., Azotobacter sp.	polysaccharide (cellulose)	cellulase	Deinema & Zevenhuizen (1971)
Pasteurella pestis	DNA	DNase	Wessman & Miller (1966)
Micrococcus halodenitrificans, Vibrio costicolus	DNA	DNase	Smithies & Gibbons (1955)
Flavobacterium	protein	pronase, trypsin chymotrypsin	Endo et al. (1976) Endo & Takahashi (1980)
Pseudomonas (phenol adapted activated sludge)	muco-polysaccharide	deflocculation enzyme produced by floc former	Tago & Aida (1977)
Pseudomonas (glycerol adapted activated sludge)	DNA	DNase I, II	Sakka et al. (1981) Sakka & Takashashi

having molecular weights of less than 6×10^6 weight were shown to be incapable of reflocculating the extracted cells, whereas double stranded DNA having molecular weights greater than 6×10^6 sucessfully flocculated extracted cells (Sakka et al., 1981; Sakka and Takahashi, 1981). A similar result was reported for activated sludge (Vallom and McLoughlin, 1984). DNA released on lysis of cells can act as an aggregation factor and can also survive breakdown or depolymerization. The key feature of bioflocculation could thus be in the aggregation factors which can cause microorganisms to flocculate, once sufficient quantities of extracellular biopolymer have accumulated at the microbial surfaces.

Besides floc formers and their extracellular polymers, filamentous bacteria are essential to the integrity of the macrostructure of the activated sludge floc. Filaments form a rigid backbone for the floc, to which flocculent zoogloeal microorganisms attach like flesh on a bone (Parker et al., 1970; 1971; 1972; Sezgin et al., 1978; Palm et al., 1978). If there are not sufficient filaments, the floc will be weak and subject to breakup into smaller aggregates in the turbulent environment of the mixed reactor.

3.2.6 Algal bioflocculation

3.2.6.1 Laboratory scale systems

The harvesting of algae from water and wastewater was investigated with synthetic organic polyelectrolytes, e.g., cationic, anionic, or nonionic polymers (Cohen et al., 1958; Golueke et al., 1964; Tenney et al., 1969). Their results

indicated that good flocculation was achieved using cationic polyelectrolytes, whereas no significant flocculation was observed with anionic or nonionic polyelectrolytes at neutral pH. Tenney et al. (1969) reported that the concentration of a cationic polyelectrolyte required for 50% flocculation of mixed algal culture reached a minimum during the declining growth phase of cultures. They thought that the initial exopolymer accumulated on the surface of the algae could promote floc formation, while excess exopolymer accumulated in later growth phase could act as a protective colloid. Pavoni et al. (1971; 1974) observed that algal flocculation directly coincided with algal exopolymer production. They found that floc formation was restricted to the declining growth phase of cultures and the surface charge of algae remained negative throughout all growth phases. They concluded that the surface coverage relationship was the main mechanism of algal bioflocculation and that reduction of surface potential was not prerequisite to bioflocculation. Avnimelech et al. (1982) reported that the extracellular polymers of Anabaena sp. could enhance floc formation and sedimentation in natural environments by forming a network structure and adsorbing clay particles.

Rao et al. (1974) suggested that the intracellular polymers released by algal cells may have an important roles in algal bioflocculation. They showed that over 80% removal of dispersed algae was achieved by bioflocculation which was induced by blending. Malis-Arad et al. (1980; 1982) observed that Chlorella sp. were clustered in an axenic culture which was maintained under alkaline conditions (pH 9.5). In this case, the

key feature of aggregated cells was an incomplete ruptured mother cell membrane which was observed to be multi-layered.

Another type of algal bioflocculation was successfully achieved in activated algae systems (activated sludge and flocculating algae-bacteria system) using laboratory scale reactors (McKinney et al., 1971 ; Humenik and Hanna, 1971; McGriff and McKinney, 1972; John and Bokil, 1979; Gupta and Rao, 1980; Nambiar and Bokil, 1981; Bokil and John, 1981; Gupta, 1985). With the continuous feeding of raw or synthetic sewage, flocculating algal-bacterial systems were developed by addition of considerable amounts of activated sludge to algae culture medium (for example, 490 mg/L of activated sludge and 260 mg/l of algae). John and Bokil (1979) reported that the optimum ratio of algae to bacteria for flocculation to take place was 60:40 (w/w). However, this system has not been reported to be completely successful so far in large scale field operations because of difficulty in balancing the quantities of algal and bacterial biomass. The mechanism of flocculation in this system is thought to be bridging and entrapment of algae by activated sludge.

The extracellular bioploymers (including intracellular polymers released by microorganisms) of algae and bacteria are thought to be important in bioflocculation which has been explained by the bridging mechanisms. It is thought that algal aggregation in the pond system is due to not only algal polymers, but also bacterial exopolymers.

3.2.6.2 Pilot and field scale systems

Oswald et al. (1978) reported that Scenedesmus spp. grown in a continuously mixed, high-rate pond system treating domestic wastewater in Manila, the Philippines, settled quickly when transferred to quiescent conditions. Subsequently, Eisenberg et al. (1981) observed that bioflocculating Micractinium spp. predominated in continuously mixed, high-rate ponds treating domestic sewage at Richmond, California. Development of flocculent, readily settleable algal cultures in both of these systems was attributed to gentle flow-mixing imparted by paddlewheels. Mixing energy requirements were low (approximately 15 kWh/ha d) because flow velocities of only 10-15 cm/s were needed. Removals of algal suspended solids by sedimentation ranged from 69-82% in the Manila study (Oswald et al. 1978). Eisenberg et al. (1981) reported that biomass removals obtained by settling the flocculent Richmond cultures in quiescent ponds averaged 79-80% during a 16-month period.

Synechocystis sp. in high-rate pond was successfully removed by algal bioflocculation which was induced by continous flow-mixing at 20-30 cm/s (Lincoln et al., 1984). DO and pH in the mixed culture declined to 5 g/m^3 and 7.0 from initial values of 18 g/m^3 and 9.0, respectively. Extracellular polymers in this case were apparantly produced by Synechocystis itself and involved in cell-to-cell bridging, because no significant bacterial component was observed microscopically.

3.3 Materials and methods

3.3.1 Laboratory experiments

Two experiments were conducted in the laboratory. In Experiment L-1, the effect of nitrogen, phosphate, organic carbon, and certain minerals (e.g., Na^+, Mg^{++}) on algal growth phase and algal bioflocculation was tested. In Experiment L-2, the effect of added bacteria and exposure to strong oxidants was examined. The experiments are summarized in Table 3-5.

3.3.1.1 Experiment L-1 - effect of nutrient media composition

4 L aliquots of anaerobic lagoon effluent (initial pH 7.5) from the University of Florida's swine research unit were adjusted to pH 9, 10, and 11 with 5 N NaOH. The aliquots, including one at pH 7.5, were sparged with nitrogen gas for 3 hours. Whitish precipitates formed in the aliquots adjusted to pH 10 and 11. All aliquots were centrifuged at 6000 x g for 20 min and the supernatants carefully decanted. pH of the supernatants was adjusted back to 7.0 with 5 N H_2SO_4. (However, the media are still referred to in terms of the original pH they were adjusted to.) Each aliquot was then divided into four 300-mL replicates.

Algal inoculum was obtained from the field high-rate pond (C-4) which was dominated by Chlorella and Monodus. The inoculum was centrifuged at 2100 x g for 10 min. Two thirds of the supernatant was decanted; the algal pellet was resuspended in the remaining supernatant. A 33 mL of inoculum was added to each flask which held 300 mL of culture medium.

Cultures were capped with porous plastic plugs and shaken at 140 oscillations/min (Lab-Line shaker table, Model 3590).

Table 3-5. Summary of laboratory bioflocculation experiments

Experiment	Variables	Conditions	Parameter mesured
L-1	Nutrient media composition	Mixing rate: 140 oscillations/min. Algal basal medium: anaerobic lagoon effluent Ranges of pH treatment pH 7.6, 9, 10, and 11 Temperature: 22 ± 1.5° C Light intensity: 7.8 watts/m^2 Reactor: 500 ml Erlenmeyer fask	Removal efficiency, Chl. a, DHA, pH, glucose, protein, nucleic acid.
L-2	Presence of native bacteria Seeding by activated sludge	Reactor: jar 11.5x11.5x20.0 cm Mxing rate: 23 s^{-1} as G Toxicant dosage (g/m^3): Cl_2; 0, 5, 25, 50 H_2O_2; 0, 10, 50, 100 Seeding dose: 2 mL actvated sludge per 2 L culture Culture: C-4 Temperature: 22 ± 1.5° C Light intensity: 11.0 watts/m^2	Removal efficiency, Chl. a, DHA, OD at 665 nm

Illumination was provided by fluorescent lights. The fluorescent light fixture consisted of five standard 40 W bulbs (F40CW, Sylvania) spaced 5.5. cm apart and located 57.5 cm above the shaker platform. The light intensity measured by a pyranometer/lightmeter (PY1617-7903, LI-Cor/LI-188, Lambda) was 7.8 watts/m^2. The experiment was conducted at room temperature (22 $\pm$ 1.5° C).

Before sampling, each group of replicate cultures was combined in a 2 L beaker and mixed throughly. After sampling, culture was equally divided among the Erlenmeyer flasks. Chlorophyl a, DHA, pH, and removal efficiency by settling were measured. Biopolymer concentration and composition were also measured.

3.3.1.2 Experiment L-2 - effect of oxidant addition and bacterial biofloc seeding

A 30 L of C-4 culture dominated by Chlorella sp. and Monodus sp. was settled for 4 hours. A 20 L of supernatant was diluted with distilled water to 40 L (TSS of 220 mg/L). Two liter aliquots of diluted culture were transfered to acrylic plastic jars with dimensions of 11.5 x 11.5 x 20.0 cm. While mixed at a G of 165 s^{-1}, the aliquots were dosed with various concentration of sodium hypochlorite or hydrogen peroxide. A standard jar test apparatus (Phipps and Bird) was used for mixing. After 2 min, mixing was reduced to 45 s^{-1} for 45 min. Mixing was continuous at a G of 23 s^{-1} for the rest of the experiment.

Four hours after oxidant addition, 2 mL of activated sludge (TSS of 7500 mg/L) from the UF wastewater treatment plant was added to half of jars. Illumination was provided by a 30 W

fluorescent light located 5 cm from jars at the side. Its light intensity measured at the surface of jars was 11.0 watts/m^2.

Samples of the mixed cultures taken 3 cm below the water surface were tested for optical density, chlorophyl a, DHA and settling efficiency.

3.3.2 Field experiments

The general experimental procedure was to fill the bioflocculation ponds with mature culture medium from the high-rate pond (C-4), monitor the channels until bioflocculation took place, and then drain and clean the ponds throughly in preparation for the next experiment. The field experiments are summarized in Table 3-6.

3.3.2.1 Experiment F-1: effect of algal biofloc seeding

Following termination of a previous experiment carried out between July and September, mixing was turned off in C-1 and sedimentation of flocculent algae was allowed for 24 hours. Simultaneously, C-2 was drained and cleaned. C-2 was filled to a depth of 11 cm with supernatant from C-1. Additional supernatant was discarded until 11 cm remained in C-1, containing most of the bioflocculated algae (major species: Synechocystis) from the previous trial. The bioflocculation ponds were brought up to a depth of 44 cm with culture medium from C-4 which was dominated by Synechocystis and Chlorella. Both C-1 and C-2 were mixed and received a volume of 0.2 m^3 fixed bed reactor effluent three times weekly. Average characteristics of fixed bed reactor effluent during the experiment were 7.58 kg/m^3 TS; 5.19 kg/m^3 VS; 10.72 kg/m^3 COD; 1.38 kg/m^3 TKN; and 1.13 kg/m^3 NH_4^+-N.

Table 3-6. Summary of field bioflocculation experiments

Experiment	Variables	Conditions	Parameter mesured
F-1	Algal biofloc seeding	Type of floc: bioflocculated *Synechocystis* Feeding source: FBR effluent Major algal species: *Synechocystis* and *Chlorella* Mixing rate: 19 cm/s	Settled volume, Chl. *a*, DHA, Total polymer, DO, pH, Visibility
F-2	Acidification	Target pH: 6.0 Feeding source: FBR effluent Major algal species: *Chlorella* and *Monodus* Mixing rate: 19 cm/s	Settling volume, pH, Chl. *a*, DHA, Total polymer, Ash free polymer, DO, Visibility, Glucose, Protein, Nucleic acid
F-3	Waste loading	Feeding source: FBR effluent Major algal species *Chlorella* and *Monodus* Mixing rate: 19 cm/s	Settling volume, pH, Chl. *a*, DHA, Total polymer, Ash free polymer, DO, Visibility

3.3.2.2 Experiment F-2: effect of acidification

A mixture of 80% culture medium from the facultative pond and 20% medium from the high-rate pond was used to fill the bioflocculation ponds to an initial depth of 30 cm. Sulfuric acid was added periodically to C-2 to reduce its pH to 6.0 or less. Both ponds were mixed and each received 0.2 m^3 fixed bed reactor effluent three times weekly. Average characteristics of fixed bed reactor effluent were 10.3 kg/m^3 TS; 7.5 kg/m^3 VS; 14.21 kg/m^3 COD; 1.49 kg/m^3 TKN; and 1.07 kg/m^3 NH_4^+-N. Dominant genera of algae in both ponds were Chlorella and Monodus.

3.3.2.3 Experiment F-3: effect of waste loading

The bioflocculation ponds were filled to a depth of 37 cm with medium from the high-rate pond. C-1 was loaded with 0.45 m^3 fixed bed reactor effluent three times weekly, whereas C-2 was not loaded. Average characteristics of fixed bed reactor effluent were 12.82 kg/m^3 TS; 8.48 kg/m^3 VS; 19.39 kg/m^3 COD; 2.55 kg/m^3 TKN; and 1.91 kg/m^3 NH_4^+-N. Both ponds were mixed.

3.4 Results and discussion

3.4.1 Laboratory experiments

3.4.1.1 Effect of media nutrient composition

Table 3-7 shows concentrations of organics, total Kjeldahl and ammonia nitrogen, and total phosphorus in pH adjusted anaerobic lagoon effluent. As expected, nitrogen concentrations decreased, but to a relatively small extent. COD and total phosphorus concentrations were initially reduced, but in the pH 11 adjusted medium their concentrations exceeded those of the control. The pH 11 medium was more transparent than the others.

Table 3-7. Organic and nutrient composition of anaerobic lagoon effluent after pH adjustment, nitrogen sparging and centrifugation. All values in g/m^3

	Medium			
	Control	pH 9 treated	pH 10 treated	pH 11 treated
COD	337	303	344	463
NH_4^+-N	313	259	220	189
TKN	325	274	236	217
Total P	5.7	4.8	4.7	6.2

This could be attributed to cell lysis at high pH. Concomitant release of soluble intracellular material with phosphorus chelating properties could explain the higher COD and P concentrations. COD:N:P ratios of the control, pH 9 treated, pH 10 treated, and pH 11 treated basal medium were 59:57:1, 63:57:1, 73:50:1, 75:35:1, respectively.

Ratios of Ca^{++} and Mg^{++} in each medium were estimated according to reported relationship between total soluble Ca^{++} and Mg^{++}, pH and equilibrium total carbonic species concentration (Benefield et al., 1982). These ratios are changed only with pH in the medium regardless of total carbonic species. The concentration of Ca^{++} in control, pH 9 treated, and pH 10 treated medium was calculated to be 250, 25, and 2.5 times greater than that in the pH 11 treated medium, respectively. The concentration of Mg^{++} in the control, pH 9 treated, and pH 10 treated medium was calculated to be 700,000, 9,000, 100 times greater than that in the pH 11 treated medium, respectively.

Temporal variations of Chl *a*, dehydrogenase activity (DHA), and pH in the experimental and control cultures are shown in Figure 3-2. Growth of algae began after 2 weeks, coinciding with declines of culture pH. Concurrently, small flocs became visible in the cultures. Microscopic observation revealed that algae were clustered each other with dead protozoan cells. The flocs were colored a dark green. By day 21, algal bioflocculation was considerably developed in all cultures (Table 3-8) and their growth rates were still high. Removal efficiencies by algal bioflocculation in the control, pH 9 treated, pH 10 treated, and

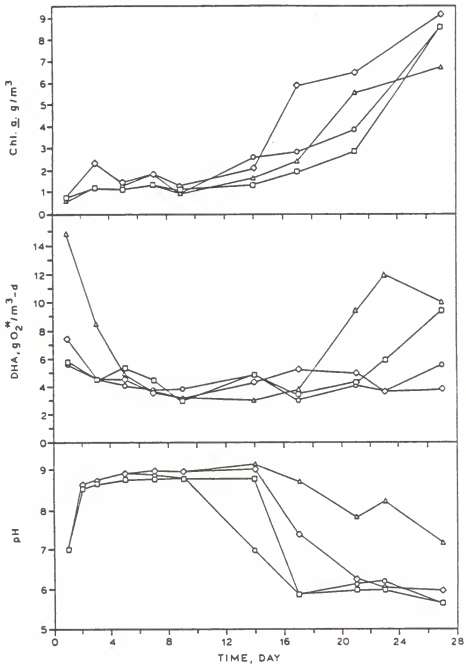

Figure 3-2. Temporal variation of chl. a, dehydrogenase activity and pH in treated anaerobic lagoon effluent. (□ = control, ○ = pH 9 treated, ◇ = pH 10 treated, △ = pH 11 treated)

Table 3-8. Comparison of algae removal efficiency by settling, total biopolymer concentration, and biopolymer composition.

Day	Treatment	Chl. removal efficiency %	Ash-free biopolymer concentration[a] g/g TSS[b]	Biopolymer composition % Polys.	% Prot.	% Nuc. acid
21	Control	80	0.203	79.5	18.9	1.5
	pH 9	85	0.172	19.5	67.0	13.3
	pH 10	71	0.071	73.0	24.6	2.4
	pH 11	46	0.050	70.0	27.2	2.8
27	Control	97	0.073	64.8	31.7	3.5
	pH 9	97	0.072	33.8	57.9	8.3
	pH 10	89	0.074	53.4	40.1	6.4
	pH 11	69	0.064	56.8	39.1	4.3

[a]Ash-free biopolymer concentration estimated as the sum of polysaccharide, potein and nucleic acid.
[b]TSS estimated as 100 x Chl. *a*.

pH 11 treated media were 80%, 85%, 71%, and 46%, repectively. By day 27, these values were further increased to 97%, 97%, 89%, and 69%, respectively.

Figure 3-3 shows the temporal variation of biopolymer concentration and composition. As indicated, there was a general upward trend in biopolymer concentration (Fig. 3-3, Top). The extent of bioflocculation of each culture at day 21 and 27 was in good agreement with the order of biopolymer concentration. The extracted biopolymers of the control and pH 9 treated media at days 21 and 27 were pale yellow. The extent of bioflocculation was also in good agreement with the intensity of yellow color in extracted biopolymer.

Glucose concentration in extracted biopolymer generally increased, whereas protein and nucleic acid concentrations were not significantly changed except in the pH 9 treated culture. Though algal removal efficiencies at day 27 and total biopolymer concentrations in the pH 9 treated and control cultures were similar, the respective biopolymer compositions were different. Glucose was higher than protein in the control, and vise versa in the pH 9 treated culture.

Algal bioflocculation directly coincided with the estimated order of Ca^{++} and Mg^{++} concentrations in the culture media.

Even though there were differences in COD/N ratios between each medium, the biopolymer production rates were not in agreement with those of other researchers (Duguid and Wilkinson, 1953; Seviour and Kristiansen, 1983; Salanitro et al., 1983; Rudd et al., 1984) who found that polysaccharide production was maximal in

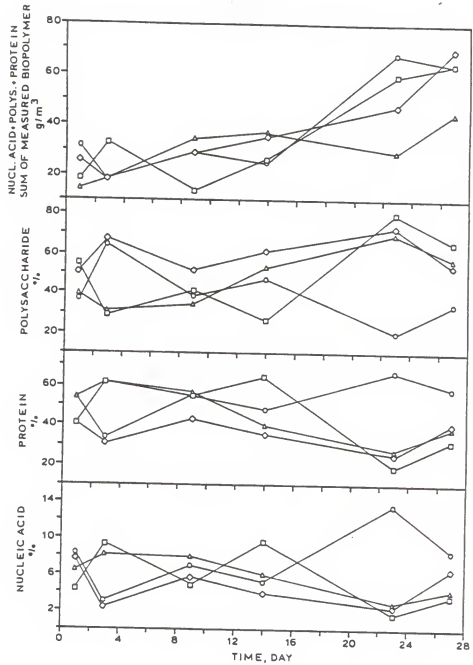

Figure 3-3. Concentration and composition of extracted biopolymer in laboratory experiment 1. (□ = control, ○ = pH 9 treated, ◇ = pH 10 treated, △ = pH 11 treated)

cultures with higher C/N ratios. Nitrogen was probably not deficient in the present experiment.

3.4.1.2 Effect of oxidant addition and bacterial biofloc seeding

The purpose of this experiment was to elucidate the roles of bacteria in algal bioflocculation.

Table 3-9 shows the temporal variation of optical density and chl. a. Optical density and chl. a concentration in cultures with activated sludge were generally higher than those without activated sludge. Cultures treated by 5 mg/L of Cl_2 and 50 mg/L of H_2O_2 had more growth than the others. This could be attributed to the reduction of protozoa. Higher oxidant dosages inhibited algal growth. Figures 3-4 and 3-5 show the variation of algae removal efficiency (RE) and dehydrogenase activity (DHA) in the oxidant treated cultures after 1.5 and 6.5 days. DHA in the seeded and non-seeded controls declined throughout the experiment. DHAs in seeded controls was always greater than those in non-seeded ones. Algal flocs were observed in the seeded controls after 1 day. Their REs gradually improved to 24% at day 6.5, and reached to 31 % by day 8. REs of the non-seeded controls were less than those in seeded controls at day 1.5, and then exceeded those of the seeded controls. By day 6.5, their RE averaged 66% and improved to 86% at day 8. These results indicate that the native floc formers which exist in algal cultures might have more important role in algal bioflocculation than the seeded ones do.

When chlorine was used, DHAs reached to about zero and REs considerably declined. This indicates that the active bacteria in algal culture could increase algal bioflocculation. Maximum

Table 3-9. Temporal variation of optical density and chlorophyll a in cultures treated with oxidants or seeded with activated sludge.

Treatment	Optical density at 665 nm				Chlorophyll a (g/m^3)			
	0 d	1.5 d	6.5 d	8 d	0 d	0.15 d	1.5 d	6.5 d
Control:								
non-seeded	0.80	0.92	0.47	0.46	5.36	5.36	5.60	4.68
seeded	0.80	0.96	1.04	1.03	5.36	5.36	5.77	5.85
5 g Cl_2/m^3:								
non-seeded	0.80	0.79	0.91	0.94	5.36	5.09	5.11	6.86
seeded	0.80	0.84	1.20	1.33	5.36	5.09	6.21	10.37
25 g Cl_2/m^3:								
non-seeded	0.80	0.75	0.63		5.36	3.31	3.26	2.82
seeded	0.80	0.76	0.68		5.36	3.31	3.28	3.13
50 g Cl_2/m^3:								
non-seeded	0.80	0.69	0.63		5.36	2.39	1.32	0.21
seeded	0.80	0.70	0.58		5.36	2.39	1.24	0.20
10 g H_2O_2/m^3:								
non-seeded	0.80	0.86	0.27	0.17	5.36	5.32	5.27	4.25
seeded	0.80	0.98	0.52	0.48	5.36	5.32	6.15	8.47
50 g H_2O_2/m^3:								
non-seeded	0.80	0.99	1.27	1.29	5.36	5.29	7.73	10.91
seeded	0.80	0.98	0.72	0.52	5.36	5.29	7.76	6.35
100 g H_2O_2/m^3:								
non-seeded	0.80	0.91	0.43	0.18	5.36	5.14	6.87	3.00
seeded	0.80	0.87	0.66	0.45	5.36	5.14	6.43	4.12

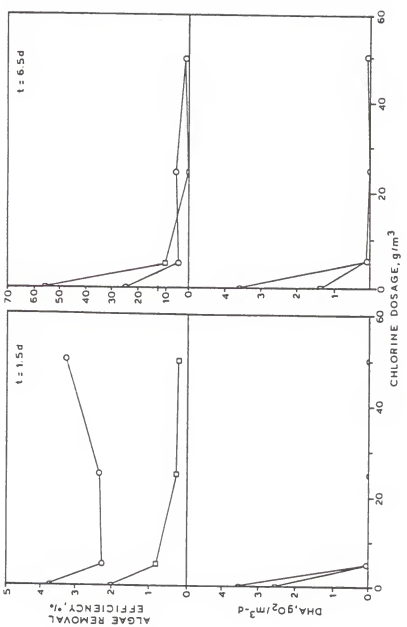

Figure 3-4. Effect of chlorine addition and bacterial biofloc seeding on algae removal efficiency and dehydrogenase activity. (□ = non-seeded, ○ = seeded)

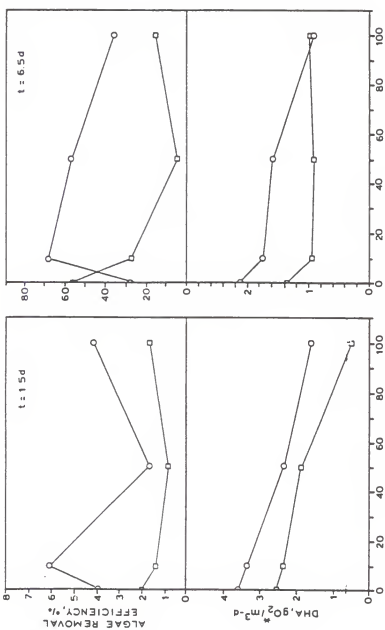

Figure 3-5. Effect of hydrogen peroxide addition and bacterial biofloc seeding on algae removal efficiency and dehydrogenase activity. (□ = non-seeded, ○ = seeded)

observed RE was 11% in the non-seeded culture treated with 5 mg/L of chlorine at day 6.5. Eventhough DHAs in chlorine treated cultures were similar, REs in seeded cultures at day 1.5 were slightly improved comparing to those in non-seed ones. This might be attributed to intracellular biopolymers which exuded from dead seeded bacteria. By day 6.5, REs in the seeded cultures treated with 25 and 50 mg/L of chlorine were slightly greater than those in non-seeded ones. High doses of chlorine bleach algae and increased algal dispersion.

With H_2O_2 added, algal bioflocculation was more improved in the seeded cultures than in the non-seeded ones. Maximum RE observed was 68% in the seeded cultures treated with 10 mg/L H_2O_2 at day 6.5. By day 6.5, RE in the seeded cultures treated by H_2O_2 was inversely proportional to dosage of H_2O_2. Unlike DHAs in cultures treated by chlorine, those in cultures treated by H_2O_2 did not reach zero at day 1.5. Their DHAs were inversely proportional to dosage of H_2O_2. By day 6.5, DHAs in non-seeded cultures treated by H_2O_2 were generally the same. DHAs in seeded cultures generally were greater than those in non-seeded cultures. Except for controls, DHA in algal cultures was somewhat related to the extent of bioflocculation.

The extent of bioflocculation was less in actively growing cultures than in the poorly growing cultures (data not shown).

3.4.2 Field experiments

3.4.2.1 Effect of algal biofloc seeding (Fig. 3-6 and 3-7)

Culture medium distributed between the two bioflocculation ponds at the start of exp. 1 was co-dominated by Synechocystis and

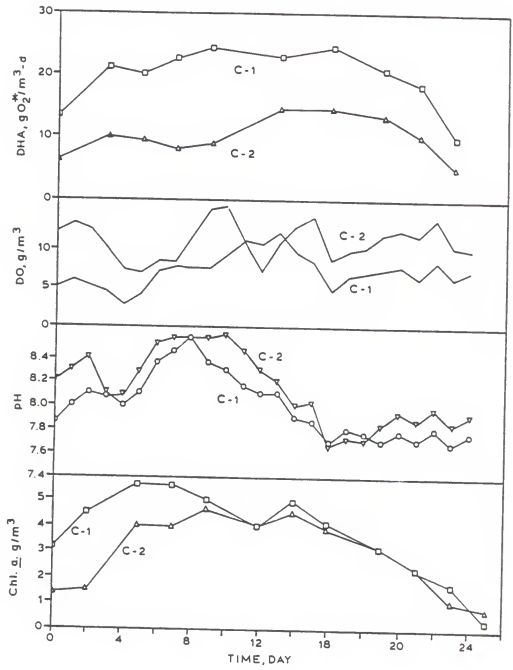

Figure 3-6. Temporal variation of DHA, DO, pH and chl. a in algal biofloc seeded (C-1) and control (C-2) cultures. Exp. F-1.

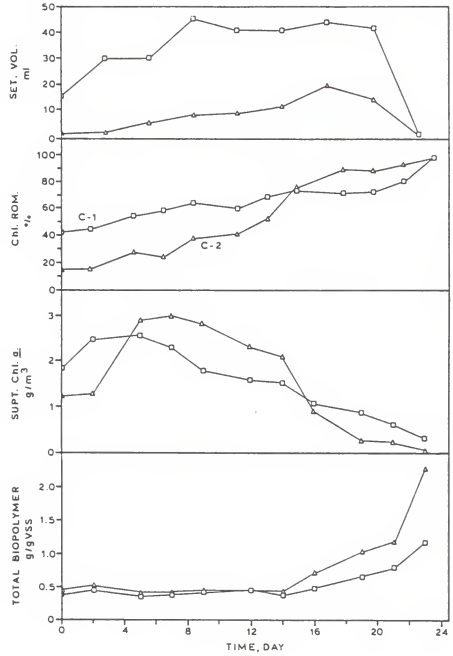

Figure 3-7. Temporal variation of settleability and total biopolymer in algal biofloc seeded (C-1) and control (C-2) cultures. Exp. F-1. (□ = C-1, △ = C-2)

Chlorella. The C-1 culture was then seeded with Synechocystis biofloc developed in the previous experiment. As a result, settleable matter was present in C-1 from the beginning of the experiment.

The floc seed increased the rate of settleable matter production in C-1 initially relative to C-2. The difference in rates eventually diminished, but the quantity of settleable matter remained greater in C-1. Algae removals in C-1 medium were also greater than in C-2 medium initially. This situation was reversed after day 16. Supernatant (after settling) Chl. a in C-1 was greater than in C-2 initially. This situation was reversed after day 5. After day 16, supernatant Chl. a in C-1 was again greater than in C-2. The volumetric concentration (data not shown) of total biopolymer was very similar in the two ponds. The mass concentration (g total biopolymer/g VSS) of total biopolymer in the two ponds was similar up to day 14. After this, medium from C-2 had the higher mass concentration.

The extracted biopolymer, after standing overnight at 4° C in ethanol, was pale yellow in appearance and sticky to the touch. These characteristics were observed throughout the experiment.

3.4.2.2 Effect of acidification (Fig. 3-8, 3-9 and 3-10)

The effect of intermittently reducing pH in culture medium dominated initially by Chlorella and Monodus was tested in exp. 2. The target pH was 6.0, but values significantly less than this (as low as 3.9) were reached after some of the acid additions. Despite experiencing extremes in pH, algal density in the experimental culture (C-2) exceeded than in the control

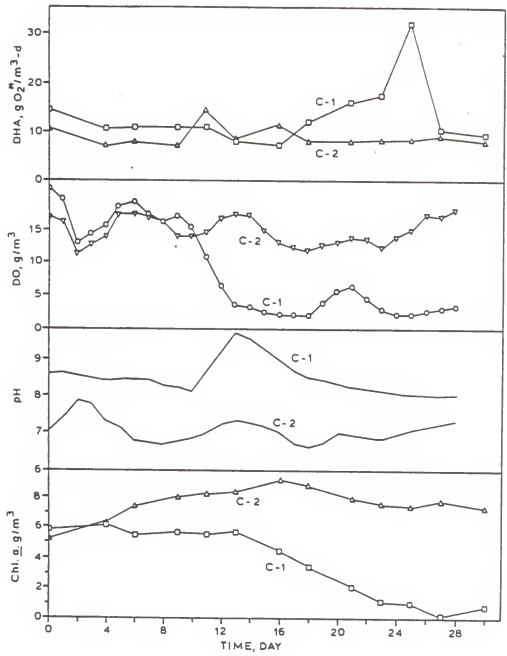

Figure 3-8. Temporal variation of DHA, DO, pH and chl. a in acidified (C-2) and control (C-1) cultures. Exp. F-2.

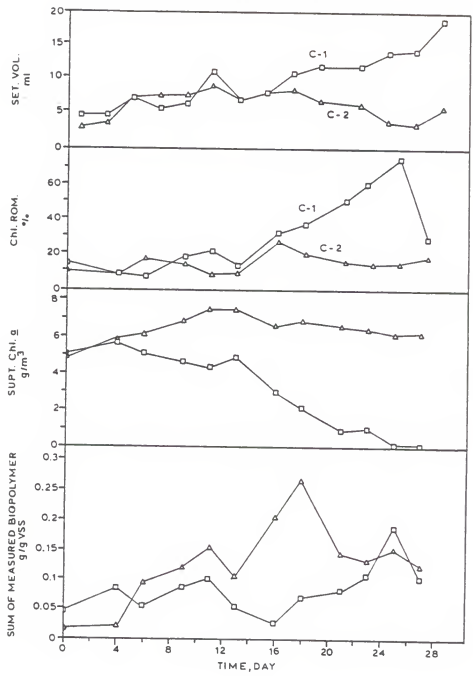

Figure 3-9. Temporal variation of settleability and ash-free biopolymer in acidified (C-2) and control (C-1) cultures. Exp. F-2. (□ = C-1, Δ = C-2)

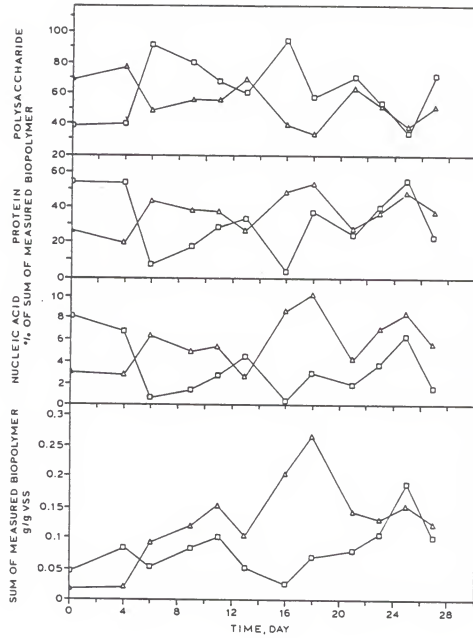

Figure 3-10. Temporal variation of biopolymer composition in acidified (C-2) and control (C-1) cultures. Exp. F-2. (□ = C-1, △ = C-2)

(C-1). Settleable matter production and algal removal were similar in the two cultures until day 13, when C-1 was dosed heavily with ammonium hydroxide for control of rotifers. Beyond this point, settleable solids and algal removal became greater in C-1 medium than in C-2 medium (Fig. 3-9). This trend was paralled by a progressive decline of chl. a in C-1. Maximum settleable solids volumes in C-1 and C-2 media were 19 mL/L and 9 mL/L, respectively. Maximum algal removals in the two media were 76% and 28%, respectively. Supernatant Chl. a in C-2 was greater than in C-1 throughout the experiment. DO was greater in the pH adjusted pond whereas DHA, pH, and visibility were less, as compared to the control (Fig. 3-8). A rapid drop of DO in pond C-1 followed closely the heavy ammonium hydroxide dosage on day 12.

The temporal variation of ash-free biopolymer (sume of measured biopolymer/VSS) of C-1 and C-2 was shown in Figure 3-9. Ash-free biopolymer concentrations of both ponds were fluctuated. At day 16, after pH in C-2 was extremely reduced to be 3.9 by sulfuric acid addition, ash-free biopolymer concentration increased sharply. This can be explained by the increase of protein and nucleic acid concentration in polymer of C-2 which might be originated from microorganisms (Fig. 3-10). Polymers extracted from C-2 were whitish in color throughout the experiment, whereas those from C-1 were white initially, then changed to pale yellow after day 15. Ash-free biopolymer concentration (g/g VSS) in C-1 progressively increased from 0.03 at day 15 to 0.19 at day 25, whereas that in C-2 was changed from

0.26 to 0.15 during same experimental periods. Concurrently, algae removal efficiency in C-1 was increased to 76%, however, algae removal efficiency in C-2 was not improved. This indicates that the yellowish extracellular biopolymer may act as binding materials between algal cells, whereas the whitish one may not. Biopolymer composition is shown in Figure 3-10.

3.4.2.3 Effect of waste loading (Fig. 3-11 and 3-12)

In exp. F-3, algal communities in the waste-loaded (C-1) and non-loaded (C-2) cultures consisted almost exclusively of Chlorella and Monodus throughout the experiment. Both cultures became visibly flocculent after approximately one week. Production of settleable matter was similar for the first three weeks, then became somewhat greater in C-2 than in C-1 (max. 54 vs. 40 mL/L) (Fig. 3-12). DO, pH and, eventually, DHA and visibility became lower in the waste-loaded culture (Fig. 3-11). Algal removals in media from the two ponds were nearly equal throughout the experiment. Also, the ash-free biopolymer concentrations in C-1 and in C-2 were smilar. Polymers of both cultures were white colored initially, then became yellowish and sticky after day 13. Concurrently, the ash content of polymers was decreased, while net biopolymer concentrations was progressively increased.

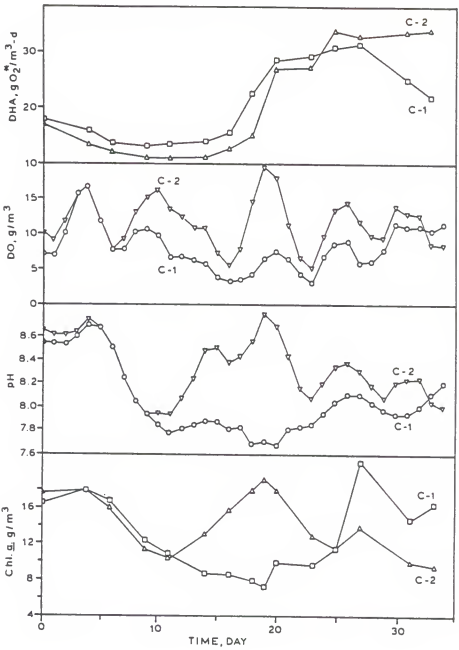

Figure 3-11. Temporal variation of DHA, DO, pH and chl. a in waste-loaded (C-1) and control (C-2) cultures. Exp. F-3.

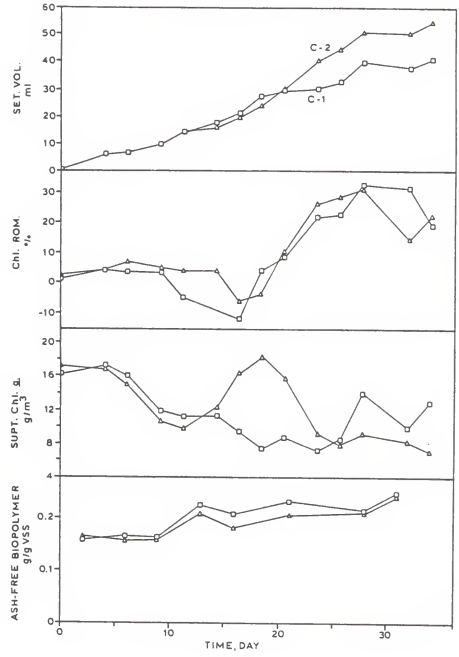

Figure 3-12. Temporal variation of settleability and ash-free biopolymer in waste-loaded (C-1) and control (C-2) cultures. Exp. F-3. (□ = C-1, △ = C-2)

PHASE II.

ALGAL FLOCCULATION WITH CHITOSAN

CHAPTER 4
EFFECT OF BIOLOGICAL AND OPERATIONAL VARIABLES ON ALGAE FLOCCULATION WITH CHITOSAN

4.1 Introduction

The work presented in this chapter focuses on algal flocculation with chitosan in high rate pond effluents. Chitosan was found to be a promising flocculant with respect to effectiveness and economy (Nigan et al., 1980; Venkataramam et al., 1980; Lavoie and de la Noue, 1983; Lavoie et al., 1984). It was found that pH and ionic strength are important to the configuration of chitosan molecules and that algal species and physiological conditions are also important variables for algal flocculation with chitosan. Pretreatment of algal cultures with ozone (Shelef et al., 1984) and chlorine (Sharma and Venkobachar, 1979) were shown to reduce flocculant dosage requirements.

The purpose of this study was to quantify the effect of pH, various chitosan formulations, algal species, extent of bioflocculation, pretreatment with strong oxidant on algae removal efficiency and chitosan dosage requirements.

4.2 Literature review

4.2.1. Production of chitosan

Chitin is an acetylated glucosamine which is structurally similar to cellulose. Chitosan is obtained from chitin by a deacetylation reaction with concentrated caustic (typically 40-50%

NaOH) at temperatures ranging from 100° to 150° C (Kohn, 1976; Averbach, 1981). The basic molecular arrangements of chitin and chitosan are shown in Figure 4-1.

Chitin is one of the most widely distributed and abundant biopolymers worldwide. The amount of chitin synthesized by marine copepods alone is estimated to be one billion tons per year (Austin et al., 1981; Berkeley, 1979). Potential commercial sources of chitin are the shells of crabs, shrimp, lobsters, krill, clams, oysters, squid, and moray. The global annual estimate of commercial chitin production from these sources is approximately 150,000 tons per year (Allan et al., 1978; Berkeley, 1979). Another source of chitin is the fermentation industry based on fungi (e.g., Aspergillus niger), which generates an estimated 800,000 tons of waste per year. This translates into approximately 200,000 tons of available chitin (Berkeley, 1979; Nicolaysen, 1980; Bartnicki-Garcia, 1969).

Wastes produced by the U.S. crustaceans industry, which consist of shrimp body peelings, shrimp head waste and crab wastes, totaled 0.11-0.14 million tons per year in 1977. Between 5300 and 7900 tons of chitin would be produced if all waste were utilized. Chitin itself is saleable as an organic slow-release nitrogen source because it decomposes slowly on the soil. Generally, a chitin-to-chitosan conversion rate is reported to be 75-80% (Kohn, 1976). Thus, total feasible chitosan production would be 4300-6400 tons per year in this country. If mass production of chitosan were achieved in U.S., chitosan product was estimated in 1976 to sell at best for as little as $2.20 and at

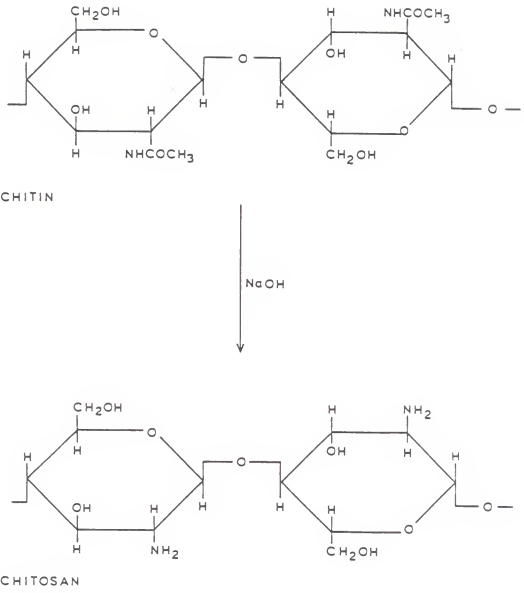

Figure 4-1. Structural formulae of chitin and chitosan.

worst for about $5.50 per kilogram. Chitosan from the Japan sold in 1976 for $4.67-5.99 per kilogram (Kohn., 1976).

4.2.2 Properties of chitosan

The properties of chitosan are sensitive to the processing parameters (Averbach and Clark, 1978). There are considerable variations in quality, depending on the methods employed (Averbach, 1981). The molecular weight of chitosan ranges from 800,000 to 1,000,000. Chitosan has a hexose backbone, like other polysaccharides, but it is unique in that it has amine groups which are positively charged, unlike most natural polysaccharides, which are either neutral or negatively charged (Muzzarelli, 1973; Lang et al., 1982). Chitosan is a strong cationic polyelectrolyte which dissolves readily in weak organic acids, such as acetic and formic, whereas it is insoluble in organic solvents (Muzzarelli, 1977).

The intrinsic viscosity determined for chitosan in solution indicates that chitosan adopts a conformation which ranges from a random coil to a more compact "quasi-globular" shape (Rha, 1984). This conformation is dictated by the degree of hydrogen bonding or electrostatic repulsion between neighboring chain segments. As for all the polyelectrolyte polysaccharides, the conformation of chitosan in solution is pH- and ionic strength-dependent, because the hydrodynamic volume is determined by the charge state of the polyion as well as by the covalent backbone structure. The hydrodynamic volume of chitosan can be manipulated by altering the intramolecular interactions between charged neighboring ions by changing pH or ionic strength. Chitosan in dilute acid at low

ionic strength is more compact than other polysaccharides due to local ordering caused by the high charge density resulting in a "quasi-globular" conformation. At high ionic strength, both electrostatic and hydrogen bonding forces are disrupted so that the chitosan conformation resembles that of the more typical random coil.

Chitosan has a more flexible backbone than other polyelectrolyte polysaccharides such as alginate or hyaluronic acid (Kienzle-Sterzer et al., 1980; 1982). The chain flexibiltiy makes it an especially useful functional material because flexibity imparts an enhanced sensitivity to charge effect in solution.

Chitosan solutions are shear thinning, with the viscosity at any given concentration, pH, and ionic strength decreasing by order of magnitute as shear rate is increased from 10 to 10^3 sec^{-1}. In the lower Newtonian region, where viscosity is independent of shear rate, the viscosity most strongly reflects the molecular conformation and the degree of chain segment interaction (Kienzle-Sterzer et al., 1982).

4.2.3 Chitosan in animal diets

Chitosan and its derivatives, which contain no protein, are not ideal animal feeds, but they seem to be digestible by rats (Landes and Bough, 1976), chickens and oxen (Rawls, 1984). Feeding experiments indicate that up to 5% chitosan can be tolerated in the diets of rats without ill effects (Landes and Bough, 1976).

Rawls (1984) reported that eight-week-old chicks fed a diet containing 10% N-acetylchitosan increased their body weight 77% during the 15-day study. However, animals fed on 10% chitosan gained only 12% of their body weight during the study. Control animals fed a regular diet gained 100% of their weight. Hens fed a diet containing 10% krill shells for seven days increased their egg laying rate 8.8% compared to their rate the week before. Hens in the same study that received a diet containing 20% N-acetylchitosan laid 16.7% fewer eggs.

4.2.4 Chitosan flocculation of microalgae

Venkataraman et al. (1980) and Nigam et al. (1980) investigated chitosan as a potential cationic flocculant, comparing it to $KAl(SO_4)_2$, $Ca(OH)_2$ and $Al_2(SO_4)_3$. Their results indicated that chitosan was the most satisfactory flocculant with respect to effectiveness and economy. Optimum dose was reported to be 50 mg chitosan/L (100 mg chitosan/g TSS) for pure cultures of Scenedesmus acutus. Nigam et al. (1980) reported that at higher chitosan doses (over 150 mg/L), removal efficiency was drastically reduced. At a concentration of 1.0 g/L absolutely no sedimentation was possible, because a fluffy agglomeration of algal cells filled the container.

Lavoie and de la Noue (1983) and Lavoie et al. (1984) showed that chitosan was a good flocculant for harvesting the freshwater alga Scenedesmus sp., the marine alga Phaeodactylum tricornutum, and concentrated outdoor microalgal cultures. Optimal dosages of chitosan for over 95% of algal removal were 20 mg/L (18-50 mg chitosan/g TSS) for freshwater algae grown in laboratory, 40 mg/L

(80 mg chitosan/g TSS) for marine algae, and 30 mg/l for concentrated outdoor algal cultures. Lavoie and de la Noue (1983) observed that algal cultures grown at relatively high temperature (25° C) required more chitosan to achieve the same degree of algal removal than algae grown at relatively low temperature (5 ° C). They attributed this phenomenon to a decrease of viscosity of the flocculation media and the production of more extracellular products at the higher temperature. Lavoie et al. (1984) found that optimum flocculation was obtained between the end of the exponential growth phase and beginning of the stationary growth phase.

Shelef et al. (1984) reported that chitosan flocculation efficiency strongly depended on the ionic strength of the culture medium. They showed that chitosan was effective as a flocculant only when the ionic strength of the culture medium was lower than 0.1. Flocculation was not observed at an ionic strength of 0.7. As the sea salt concentration increased, the intrinsic viscosity value decreased rapidly from 2000 mL/g to a constant value of 100 mL/g. They concluded that the polymer changes from an extended linear configuration to a randomly coiled one. With 2.5 mg/L of chitosan as flocculant aid, removal efficiencies were significantly improved using ferric chloride as the primary flocculant. They also found that with pre-ozonation, the dosage requirement of ferric chloride was reduced by 4-5 times.

pH optima of 7.0 (Lavoie et al., 1984), 8.4 (Venkatraman et al., 1980), and 7.5-8.5 (Nigam et al., 1980) have been reported.

The variability of optimum pH may result from different methods employed for chitosan production.

4.3 Materials and methods

4.3.1 Standard jar test procedure

Samples taken from the high-rate pond (C-4) or bioflocculation ponds (C-1 or C-2) were analyzed for TSS concentration and then adjusted to pre-set pH values with 8 N H_2SO_4 or 5 N NaOH. Jar tests were carried out according to the standard procedure described in Appendix A. The sample size was 1 L per jar.

4.3.2 Preparation of chitosan solution

Approximately 11 g of chitosan flakes were added to 1 L of 1% acetic acid solution. This mixture was stirred by a magnetic stirrer until dissolution occurred (30-60 min). Large insoluble particles were removed by filtering the solution through a stainless steel mesh with 2 mm opening. The actual chitosan concentration was calculated on the basis of ash free dry weight. This was obtained by drying 10 mL of the chitosan solution for 24 hours, weighing, ashing the chitosan solids at 550° C for 15 min, and weighing again. The difference between these weights gave the ash free dry weight. The ash free dry weight of chitosan formulations ranged from 94.6-96.9% of the dry weight. The different chitosan formulations are characterized in Table 4-1.

4.3.3 Experimental procedures

4.3.3.1 Effect of pH, chitosan formulation and settling time: Experiment L-4

Except as noted, the field and laboratory experiments were carried out with chitosan made from snow crab shells (Protan, lot

123-121-03) at a stock solution strength of 1.0% (ash-free dry weight basis). Before conducting further experiments, pH optimization was carried out. Aliquots of C-4 culture dominated by Chlorella and Monodus were adjusted to pH values in the range of 2 to 12.

Four different formulations of chitosan were used to determine the effect of chitosan characteristics on algal flocculation: (1) high viscosity (Protan lot #, 054-022-01), (2) medium viscosity (Protan lot # 044-021-01), (3) low viscosity (Protan lot # 044-021-01), and (4) medium viscosity (Protan lot # 123-121-03). The formulations are characterized in Table 4-1. The culture was taken from C-4 at a time when the dominant algae were Chlorella and Monodus. As 1% chitosan solutions were too viscous, they were diluted to 0.1% (w/w) with distilled water. This enabled more precise dosing. The alternative chitosan formulations were evaluated at a dosage of 15 mg chitosan/g TSS.

Optimum settling time was determined by comparing removal efficiencies of flocculated samples taken after 1, 5, 10, 15, 20, 25, and 30 min of quiescent sedimentation. The chitosan dosage was 32 mg/g TSS.

4.3.3.2 Effect of mixing in the flocculation test: Experiment L-5

A sample of the C-4 culture medium was adjusted to pH 6.5 with 8 N H_2SO_4. The dominant algal genera were Chlorella and Monodus (70% of biovolume) and Synechocystis (30% of biovolume). Rapid mixing (125 rev/min; $G = 165\ s^{-1}$) times of 10, 30, 60 and 90 seconds, followed by slow mixing at 20 rev/min ($G = 13\ s^{-1}$) for 15 min, were tested. The chitosan dosage was 61 mg/g TSS. Slow

Table 4-1. Chitosan formulations used in flocculation experiments

	No.1	No.2	No.3	No.4
Lot number	044-021-01	054-022-01	035-280-08	123-121-03
Production date	8/20/85	1/15/85	10/10/85	3/10/85
Grade	premium	premium	premium	premium
Source of chitosan	king crab	king crab	Dungeness crab	Snow crab
Viscosity in 1% solution	512 cps	3820 cps	57 cps	590 cps
Deacetylation	84.7%	>70%	92.5%	>70%
Insolubles	0.03%	1.92%	0.32%	6.1%
Moisture	0.1%	13.4%	10.0%	12.6%
Ash	0.1%	0.29%	0.21%	0.99%

mixing speeds of 10, 20, 30, 40, 50 and 60 rev/min (G = 4.6, 13, 22, 34, 47 and 61 s^{-1}, respectively) for 15 min., following one minute of rapid mixing at 125 rev/min (G = 165 s^{-1}), were also tested. The chitosan dosage was 61 mg/g TSS. Finally, slow mixing times of 5, 10, 15, 20, 25 min at 20 rev/min (G = 13 s^{-1}), following one minute of rapid mixing at 125 rev/min (G = 165 s^{-1}) were evaluated. The chitosan dosage was 45 mg/g TSS. Supernatant was taken for evaluating removal efficiency after 30 min settling.

4.3.3.3 Effect of pretreatment with strong oxidants: Experiment L-6

Two liters of aliquots of C-4 culture, dominated by Chlorella and Monodus, were transfered to macro gator jars. While the aliquots were mixed at 125 rev/min (G = 165 s^{-1}) using the standard jar test apparatus, selected dosages of strong oxidants (sodium hypochlorite or hydrogen peroxide) were added. Two minites of additional rapid mixing was given. Slow mixing at 50 rev/min (G = 47 s^{-1}) for 45 min followed. Dosages of chlorine and hydrogen peroxide were 1, 3, 5, 10, 15, and 20 mg/L. The pretreated aliquots were then flocculated with chitosan (26 mg/g TSS) using the standard jar procedure described in Appendix A.

4.3.3.4 Effect of algal species: Experiment L-7

The chitosan dosage requirements of culture medium dominated by Chlorella and Monodus were compared to the dosage requirements of medium dominated by Synechocystis. The standard jar test procedure described in Appendix A was employed. Trials with Chlorella and Monodus were conducted in late July 1985, whereas those with Synechocystis were conducted in late September 1985. The TSS of Synechocystis dominated culture medium was

approximately one half of that of the Chlorella/Monodus dominated culture medium (400 vs 880 mg/L).

4.3.3.5 Effect of pond operation: Experiments F-4, F-5, F-6 and F-7

The field scale biofloculation ponds (C-1 and C-2) were operated to test the effect of waste loading, flow mixing velocity, and biofloc seeding on algal biofloculation. During these trials, samples of C-1 and C-2 medium were tested to determine chitosan dose requirements. The standard jar test procedure described in Appendix A was employed. Feed source characteristics, organic loading rate and rainfall at the high-rate pond are given in Table 4-2. The pond operational conditions employed in this series of experiments are described next.

Experiment F-4. The biofloculation ponds were filled to a depth of 37 cm with medium from the high-rate pond which was dominated by Chlorella and Monodus. C-1 was loaded with 0.45 m^3 fixed bed reactor effluent three times weekly, whereas C-2 was not loaded. Both C-1 and C-2 were mixed at 16 cm/s.

Experiment F-5. Culture medium from the facultative and high-rate ponds which was dominated by Chlorella and Monodus was used in relative proportions of 58% and 42%, respectively, to fill the biofloculation ponds to an initial depth of 46 cm. C-1 was mixed at 14 cm/s whereas C-2 was mixed at 30 cm/s. Both C-1 and C-2 received 0.45 m^3 fixed bed reactor effluent three times weekly.

Experiment F-6. The biofloculation ponds were filled to an initial depth of 35 cm with culture medium from the high-rate pond which was dominated by Synechosystis. C-1 was mixed at 14 cm/s

Table 4-2. Feed source characteristics, organic loading rate, and rainfall at the high-rate pond.

Trial number	Feed source	Characteristics of feed source					Loading rate m^3/week	Rain fall cm
		TS	VS	COD g/m^3	TKN	NH_4-N		
1	FBR	12,820	8,480	19,390	2,550	1,910	1.35	15.2
2	FBR	4,080	2,160	9,510	1,590	1,260	1.35	6.6
3	FBR	3,440	1,580	2,760	1,020	910	1.35	0.1
4	-						-	2.3

and C-2 was mixed at 30 cm/s. Both C-1 and C-2 received 0.5 m^3 fixed bed reactor effluent three times weekly.

Experiment F-7. Culture media from C-4 which was dominated by Chlorella and Monodus were used to fill the bioflocculation ponds to an initial depth of 25 cm. A 380 L of activated sludge from the Main Street Sewage Treatment Plant, Gainesville, was added to C-2. The total suspended solids concentration of the activated sludge was 7000 g/m^3. The bioflocculation ponds were mixed at a velocity of 16 cm/s.

4.4 Results and discussion

4.4.1 Effect of pH, chitosan formulation and settling time

pH had a profound effect on algae flocculation with chitosan (Fig. 4-2). Optimal removal efficiency with crab chitosan was at pH 3. A secondary optimum was observed at pH 6.5, although similar performance was found over the pH range of 5-7. Similar trends were exhibited with the four different chitosan formulations (Fig. 4-2, bottom). These relationships may be due in part to the changing conformation of chitosan molecules in solution with pH. Another effect of pH is to change the degree of ionization of active sites of algae. In the pH range of maximal algae removal (pH 3-4) the algal zeta potential is at a minimum (Tenney et al., 1969; Golueke and Oswald, 1970). Lavoie and de la Noue (1983) reported that the optimum pH for chitosan flocculation depended on the physiological condition of the algae and on the dominant algal species. The improvement in removal efficiency at pH exceeding 10 is probably due to entrapment of algae in Ca^{+2} and Mg^{2+} precipitates, especially $Mg(OH)_2$. This phenomenon was

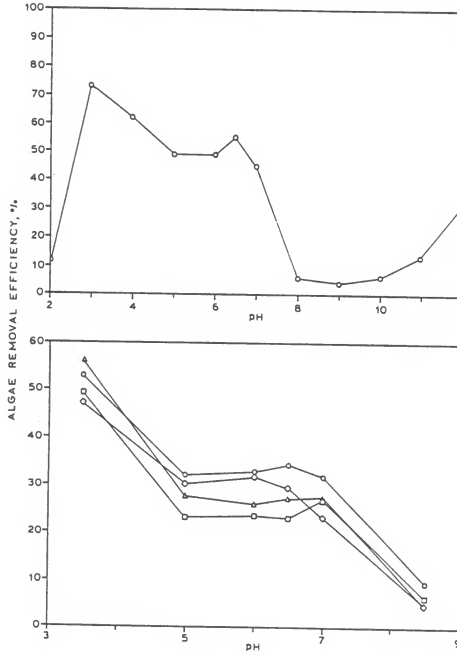

Figure 4-2. Effect of pH on chitosan flocculation. Top - snow crab chitosan; bottom - alternative chitosan formulations. Top - snow crab chitosan; botton - ○ = king crab, high viscosity, □ = king crab, medium viscosity, △ = snow crab, medium viscosity, ◇ = dungeness crabs, low viscosity. Chitosan dosage was 26 mg/g TSS.

observed by several researchers (Wachs, 1969; Folkman and Wachs, 1973; Ayoub and Koopman, 1986; Ayoub et al., 1986).

A settling time as brief as 10 min was sufficient (Figure 4-3). Longer settling times did not improve algae removals.

4.4.2 Effect of mixing in the flocculation test

Figure 4-4 shows the effect of fast and slow mixing times on algae flocculation with chitosan. Algae removal efficiency peaked at a rapid mixing time of 60 seconds (Fig. 4-4, top). Algae removal efficiencies gradually increased as the time of slow mixing was extended from 5 to 25 min (Fig. 4-4, bottom). Beyond 15 min, however, the improvement was negligible. Figure 4-5 shows that chitosan flocculation was considerably affected by slow mixing speed. Maximum algae removal was at a speed of 50 rev/min ($G = 47\ s^{-1}$).

4.4.3 Effect of pretreatment with strong oxidants

Figure 4-6 shows the relationship between oxidant dosage and algae removal efficiency with a constant chitosan dosage of 28 mg/g TSS. Removal efficiencies were increased by pretreatment with either chlorine or hydrogen peroxide. Maximum removal was achieved with 5 mg/L of chlorine. With hydrogen peroxide, removal efficiency was improved at a dosage of 1 mg/L. Some researchers (Sharma and Venkobachar, 1979; Shelef et al., 1984) found that pretreatment with chlorine or ozone could reduce flocculant dosage requirement and improve flocculation. Results of this study are in good agreement with their results. It can be postulated that pretreatment with strong oxidant can oxidize exopolymers surrounding algal biomass, enhancing vacant sites on the algal

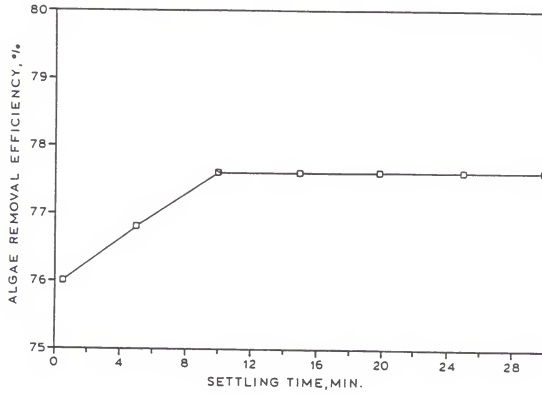

Figure 4-3. Effect of settling time on algae removal efficiency. Chitosan dose = 32 mg/g TSS.

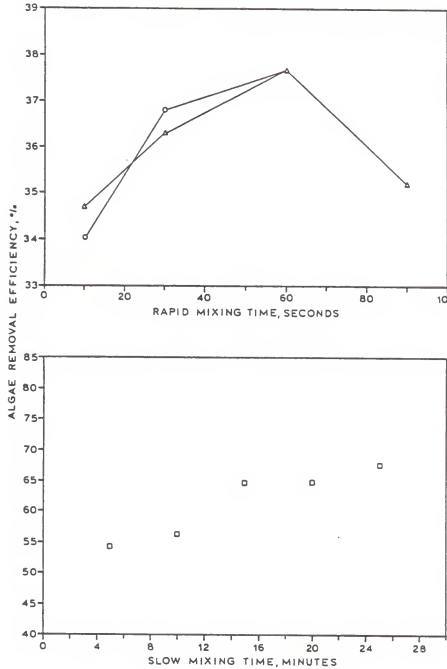

Figure 4-4. Effect of rapid and slow mixing times on chitosan flocculation. Top - rapid mixing at 125 rev/min, chitosan dosage = 45 mg/g TSS, ○ = trial 1, △ = trial 2; bottom - slow mixing at 20 rev/min, chitosan dosage = 61 mg/g TSS.

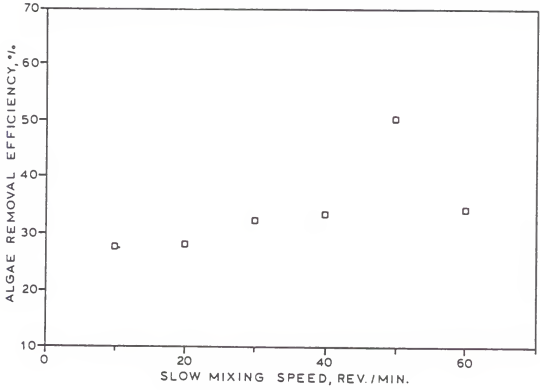

Figure 4-5. Effect of slow mixing speed on chitosan flocculation. Slow mixing time = 15 min, chitosan dosage = 45 mg/g TSS.

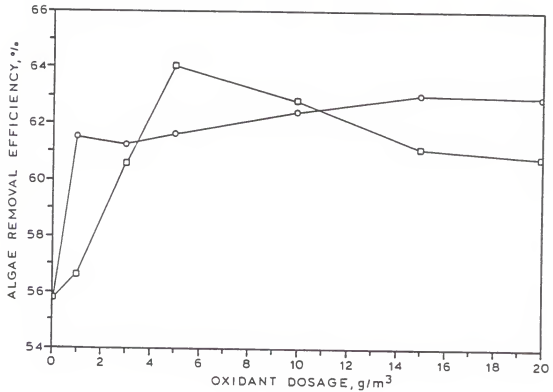

Figure 4-6. Effect of pretreatment with varying oxidant dosages on algae removal by chitosan flocculation. Chitosan dosage = 28 mg/g TSS. □ = chlorine, ○ = hydrogen peroxide.

surface. Shelef et al. (1984) observed that pretreatment by 2.5-4.5 mg ozone/L reduced the required ferric chloride dosage from 250 to 25 mg/L with the marine microalga Isochrysis galbana.

4.4.4 Effect of algal species

Figure 4-7 shows the variation of removal efficiency as a function of chitosan dosage for culture media dominated by Chlorella and Synechocystis, respectively. The chitosan dosage required to remove Synechocystis was significantly greater than that required to remove Chlorella. This difference can in part be attributed to the difference in size between the species. Typical Synechocystis diameter is 1.4-1.6 μm, whereas Chlorella typically have a diameter of 4-5 μm. At any given concentration, the total surface area of Synechocystis would be approximately 9 times the total surface area of Chlorella.

Besides the difference in size between these species, the difference of ionic strength between the cultures and the extent of loose biopolymer accumulation on algal surfaces could affect dosage requirements. Because the feed source was not changed, the ionic strength of the two culture media should have been similar. Comparing the ratio of ash free biopolymer to TSS between two cultures (initial 5 days average of Experiment F-4 and Experiment F-6), that of Synechocystis was somewhat higher (about 20%) than that of Chlorella. Thus, the loose biopolymer accumulation on algal surfaces might also have affected the dosage requirements.

Relationships of algae removal efficiency versus flocculant dosage had the same general shape as the curves depicted in Figure 4-7. These relationships can be approximated by the expression:

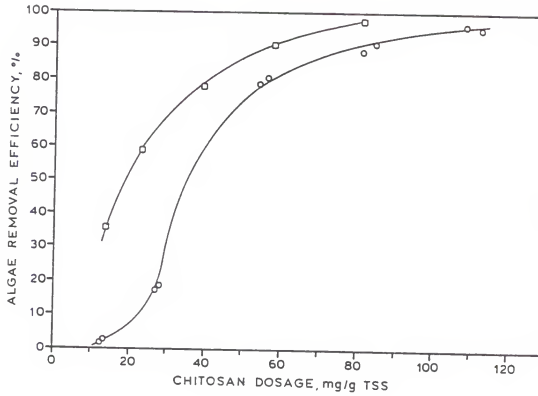

Figure 4-7. Effect of algal species on chitosan dosage requirements. □ = Chlorella, ○ = Synechocystis.

$$E_r = a/C + b \qquad (6\text{-}1)$$

where E_r = algae removal efficiency, a and b are arbitrary constants, and C = flocculant dosage (mg/g TSS). The data in Figure 4-7 are replotted according to this expression in Figure 4-8. Lines of best fit were found by least squares linear regression and used to give the flocculant dosage required for 50% algae removal (DR_{50}). The DR_{50}s were 18 mg chitosan/g TSS for Chlorella and 39 mg chitosan/g TSS for Synechocystis.

4.4.5 Effect of pond operation

4.4.5.1 Waste loading

Figure 4-9 shows the temporal variation of TSS, settleable volume, ash-free biopolymer, and chitosan dosage required for 50% algae removal in experiment F-4. The DR_{50} differed slightly but consistently between the two ponds. That of C-1, which was loaded by anaerobic fixed bed reactor effluent, was consistently 1-2 mg chitosan/g TSS less than that of C-2, which was not loaded. The DR_{50}s in both ponds gradually decreased while the extent of bioflocculation as measured by the accumulation of settleable matter increased.

Biopolymer accumulated gradually throughout the trial. The nature of the biopolymer was also variable. Initially the extracted material (in ethanol) was milky in appearance and had no adhesive properties. After day 11, the extracted biopolymer was yellow in color and sticky to the touch. The change in the

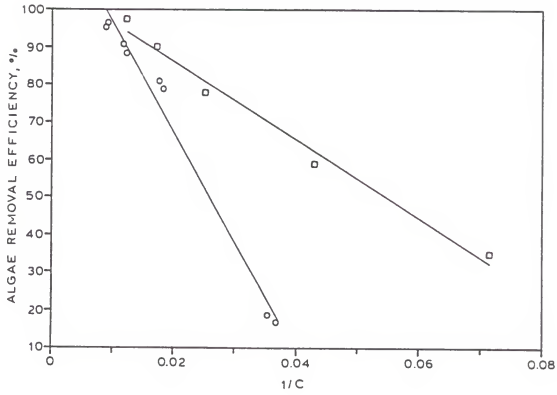

Figure 4-8. Reciprocal plots of chitosan dosage versus algae removal efficiency. □ Chlorella: $E_r = -1036.2 \times 1/C + 107.0$; r = 0.9846 ○ Synechocystis: $E_r = -2936.8 \times 1/C + 126.0$; r = 0.9824

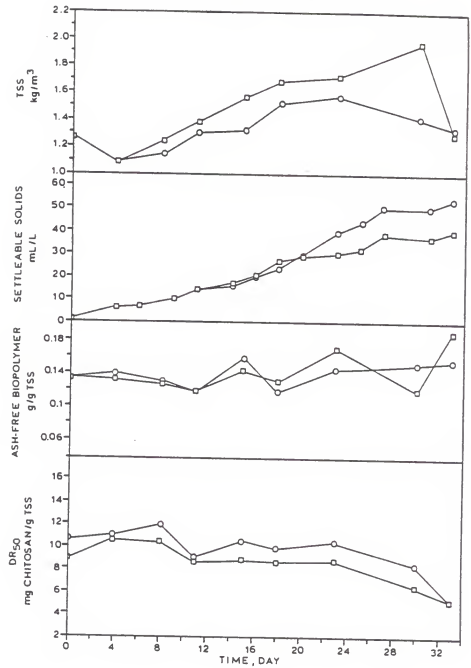

Figure 4-9. Temporal variation of total suspended solids, settleable solids, ash-free biopolymer, and chitosan dosage requirements in waste-loaded and non-loaded ponds (Experiment F-4). □ = waste-loaded, ○ = non-loaded

quality of extracted biopolymer coincided with a drop in the chitosan dosage requirements.

4.4.5.2 Flow mixing velocity

Flow mixing velocities of 14 cm/s in C-1 and 30 cm/s in C-2 were employed during experiment F-5. Chlorella and Monodus were co-dominant initially and remained so throughout the trial. In experiment F-6, the flow mixing velocity was 30 cm/s in C-1 and 14 cm/s in C-2. Synechocystis was dominant initially, but was eventually replaced by Chlorella and Monodus. Both ponds in the latter trial suffered from rotifer (Brachionis) infestations. The color of C-1 culture medium was changed from turquoise to olive green by day 7, whereas C-2 medium remained turquoise through day 12.

The temporal variation of TSS, settleable volume, ash-free biopolymer and chitosan dosage required for 50% algae removal during experiment F-5 is shown in Figure 4-10. The accumulation of settleable matter was more extensive in the faster mixed of the two ponds. The difference between ponds became greater as the experiment progressed. DR_{50}s in culture medium from the faster mixed pond were consistently less than those of the slower mixed pond. This difference also became progressively greater. Suspended solids in the faster mixed pond had a consistently lower content of ash-free polymer than suspended solids from the slow mixed pond.

The mechanism of the effect of mixing on chitosan dosage requirements can be postulated as follows. At the higher mixing intensity, the conformation and orientation of surrounding

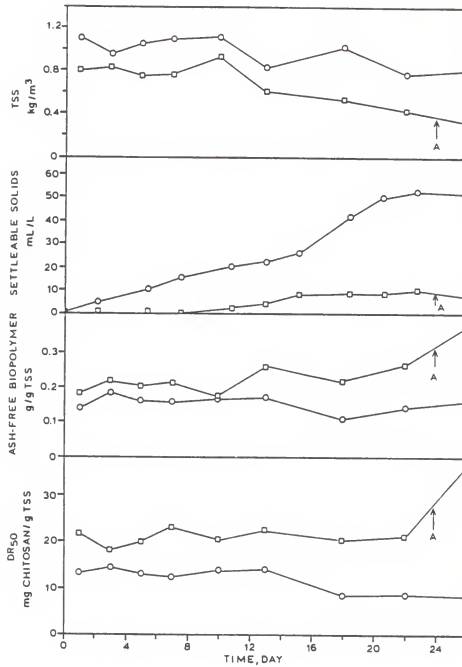

Figure 4-10. Temporal variation of total suspended solids, settleable solids, ash-free biopolymer and chitosan dosage requirements in fast and slow mixed ponds (Experiment F-5). ○ = fast mixed, □ = slow mixed, A = mixing stopped.

extracellular biopolymer is changed from a globular type to a finger like type by the increase of shear forces increasing the number of vacant sites on the algal surfaces, and enabling the relatively strong and rigid chitosan molecules to adsorb directly to the algae. The higher ratio of ash-free biopolymer to TSS in C-1 medium (the slow mixed pond) contrasts with its lesser extent of bioflocculation and higher DR_{50}s. This indicates that loose biopolymer can inhibit chitosan flocculation by adsorbing chitosan. Extracellular polymers generally carry a negative charge, making interaction with the positively charged chitosan likely. Conversely, the "sticky" extracellular biopolymer could very well increase the effectiveness of chitosan by bridging between algal cells. The ash-free biopolymer content in C-1 medium increased sharply after mixing was stopped on day 22 (Point A in Fig. 4-10). This was paralled by a sharp increase in the DR_{50} for C-1.

Again in experiment F-6, the faster mixed culture had the greatest accumulation of settleable matter (Fig. 4-11). Unlike the prior trial, the suspended solids in this culture were also higher in content of ash-free biopolymer. Chitosan dosage requirements in culture media from the two ponds were similar. They declined in the first 24 hours of mixing, but increased again beyond day 4. The results may indicate that with cultures dominated by Synechosystis, a flow mixing velocity greater than 14 cm/s will not reduce chitosan dosage requirements. It was noteworthy that DR_{50} was rapidly reduced from 39 to 29 mg

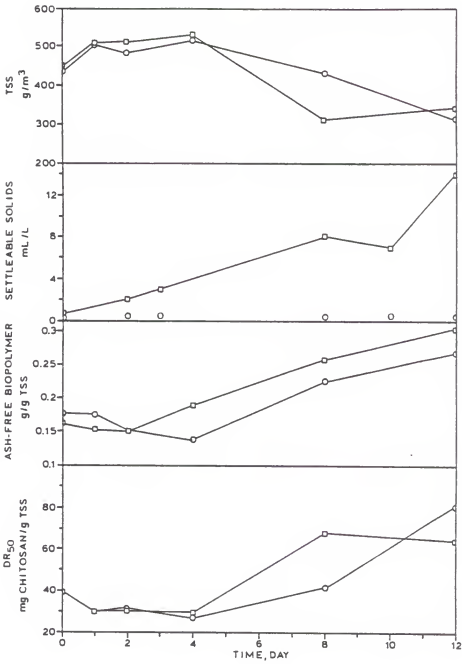

Figure 4-11. Temporal variation of total suspended solids, settleable solids, ash-free biopolymer and chitosan dosage requirements in fast and slow mixed ponds (Experiment F-6). □ = fast mixed, ○ = slow mixed

chitosan/g TSS (a decrease of one-fourth) after only 24 hour mixing.

4.4.5.3 Bacterial biofloc seeding

The temporal variation of TSS, settleable volume and chitosan dosage requirements are shown in Figure 4-12. Culture medium was transfered from the high-rate pond (C-4) into C-1 and C-2 (bioflocculation ponds) one week before mixing was started in the bioflocculation ponds. Particulate matter which settled during this period was resuspended with the start of mixing, causing a sharp increase in TSS. Addition of activated sludge to C-2 increased the TSS concentration further. The DR_{50} in C-2 was consistently less than that in C-1 during the experiment. This indicates that the activated sludge itself was more amenable to chitosan flocculation than the algae. The chitosan dosage requirements of both cultures dropped sharply upon the start of mixing, probably reflecting the influence of the resuspended matter. The continued trend of decreasing DR_{50}s paralled the increase of settleable volume in the cultures.

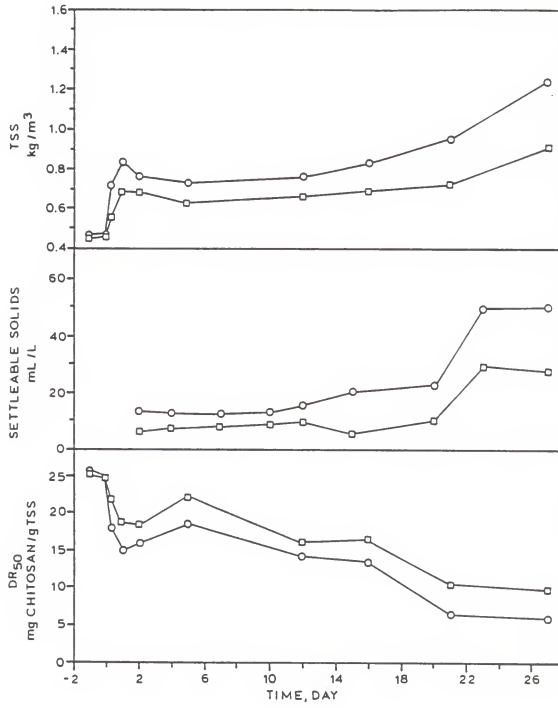

Figure 4-12. Temporal variation of total suspended solids, settleable solids, and chitosan dosage requirements in biofloc seeded and non-seeded ponds (Experiment F-7).
○ = biofloc seeded, □ = non-seeded

CHAPTER 5
RELATIONSHIP OF GROWTH PHASE AND TURBULENCE IN LABORATORY SCALE ALGAL CULTURES TO CHITOSAN DOSAGE REQUIREMENTS

5.1 Introduction

Tenney et al. (1969) found that algal growth phase influenced the dosage of cationic polymer required to attain a given degree of flocculation. The minimum requirement was observed immediately after cessation of exponential growth. Optimum flocculation was obtained at a surface coverage by added polymer of 50%, with stabilization and restabilization occurring at lower and higher surface coverage ratios, respectively. Other investigators (Avnimelech et al.,1982; Lavoie and de la Noue, 1983; Lavoie et al., 1984) also observed that the flocculation tendency was higher during the declining growth and death phases or when a culture is under stress. Besides algal physiology, operational conditions of ponds may be also important. Previous results of this study (chapter 4) suggest that chitosan dosage requirements are affected by mixing intensity in the culture. The objectives of the work presented in this chapter were to evaluate the effect of growth phase and culture mixing on chitosan dosage requirements.

5.2 Materials and methods

5.2.1 Flocculation test

The micro jar test apparatus described in Appendix A was employed. The paddles which had a height of 1.5 cm and length of

1.75 cm were used. Samples were analyzed for TSS concentration and then adjusted to pH 6.5 with 8 N H_2SO_4. The sample size was 100 mL in each jar.

5.2.2 Experimental procedures

This study consisted of three experiments. In the first, the effect of algal growth phase was evaluated. In the second and third experiments, the effects of mixing intensity and its duration in the algal culture were examined.

5.2.2.1 Effect of algal growth phase: Experiment L-8

Alum flocculated anaerobic lagoon effluent served as a basal medium. Effluent obtained from the anaerobic lagoon was flocculated with aluminum sulfate ($Al_2(SO_4)$ $18H_2O$) to remove Thiopedia rosea and other suspended solids. The dosage was 1.2 g alum/g TSS, as suggested by Freedman et al. (1983). Alum was added as a 14% solution. The flocculation procedure for removing particulate matter from the lagoon effluent consisted of 1 min rapid mixing at 125 rev/min ($G = 165\ s^{-1}$), 1 hour of slow mixing at 12 rev/min ($G = 6\ s^{-1}$) and 4 hours of quiescent settling. Flocculation was carried out in macro gator jars filled to a liquid volume of 2 L. The supernatant was carefully decanted from individual jars at the end of the settling period, and combined in a 20 L glass bottle, giving an initial liquid volume of 18 L. This supernatant was used as culture medium. No inoculum was added. Initial optical density of culture medium at 665 nm was 0.063. The medium was completely mixed by aeration initially. As mixing intensity declined due to the reduction of culture volume, settled solids increased. These were excluded from samples.

Illumination was provided by fluorescent lights. The fluorescent light fixture consisted of five standard 40 W bulbs (F40CW, Sylvania) spaced 5.5 cm apart and located 45 cm above the culture. Dominant algal genera were Chlorella and Monodus. Approximately 700 mL of sample was removed each day for the floccuation test and determination of biopolymer. After 20 days, the final culture volume was approximately 5 L. Total suspended solids (TSS), optical density at 665 nm, total and ash free biopolymer, and chitosan requirement for 50% algal removal were measured daily.

5.2.2.2 Effect of mixing in the culture vessel: Experiments L-9A and L-9B

In experiment L-9A, alum flocculated anaerobic lagoon effluent was used as the culture medium. Flocculation was carried out according to the procedure just described. 36 L of flocculated supernatant was equally divided into two 20 L glass bottles. Illumination and mixing was provided fluorescent lights and aeration described in section 5.2.2.1. Cultures dominated by Chlorella and Monodus. After 12 days, the cultures were combined in a 40 L, plastic container. The TSS and optical density at 665 nm of the combined culture medium were 177 g/m^3 and 0.441, respectively. With the culture completely mixed, 1.8 L aliquots were transferred to 2 L glass beakers. One group of four beakers was not mixed, the second group was mixed at 20 rev/min ($G = 7\ s^{-1}$) by a standard jar test apparatus, and the third group was mixed at 40 rev/min ($G = 19\ s^{-1}$) by another jar test apparatus. Values of mean velocity gradient (G) were calculated from rotor speed (in rev/min) using the calibration of Camp (1968). Illumination was provided by a 40 W fluorescent light positioned 5 cm from side of

the beakers. After complete mixing of each beaker except unmixed cultures, 600 mL of sample was taken from each group (about 150 mL per beaker). Settled solids were included in the samples of the mixed cultures. Algae removal by gravity settling was tested by 30 min of quiescent sedimentation. Total suspended solids (TSS), pH, optical density and chitosan dosage requirement for 50% algae removal were measured daily.

To begin experiment L-9B, culture media from experiment L-9A, after 5 days, were combined in 20 L glass jar. Illumination was provided using the set up described in section 5.2.2.1. No mixing was provided. After one day of settling, the supernatant was carefully siphoned to another glass vessel. With complete mixing, 1.8 L aliquots of the supernatant were transfered to 2 L glass beakers. One group of 3 beakers was not mixed, one was mixed at 20 rev/min ($G = 7\ s^{-1}$) and one was mixed at 40 rev/min ($G = 19\ s^{-1}$). Sampling was carried out according to procedure just described. Total suspended solids (TSS), pH, optical density and chitosan dose required for 50% algae removal were determined daily.

5.3 Results and discussion

7.3.1 Effect of growth phase

The temporal variation of TSS, ash-free biopolymer and chitosan dosage required for 50% algal removal are shown in Figure 5-1. The TSS concentration increased rapidly during the first 4 days and then reached a plateau. After day 8, it increased again through day 14. The TSS was 73 g/m^3 at day 2 and 230 g/m^3 at day 14. TSS subsequently decreased to 155 g/m^3 at day 20. The

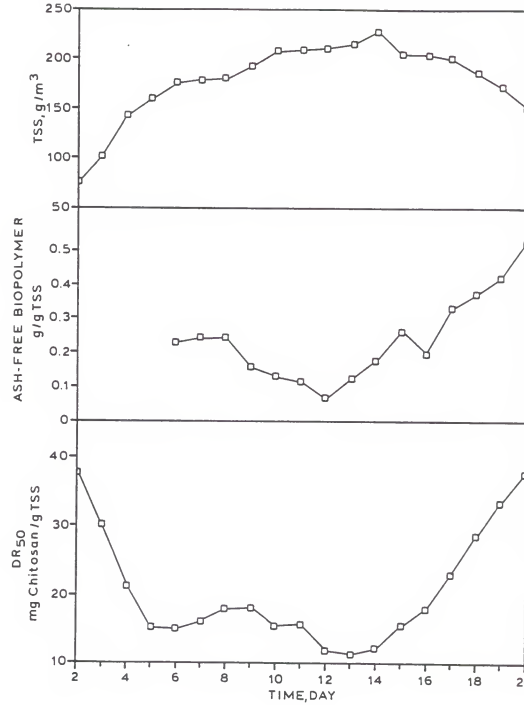

Figure 5-1. Temporal variation of total suspended solids, ash-free biopolymer and chitosan dosage requirement in batch algal culture (Exp. L-8).

decrease of TSS may have been due to a gradual reduction of mixing intensity in proportion to the reduction of culture depth, rather than algal death or decay. When the culture volume was over 7 L, complete mixing was provided by aeration. However, as culture volume declined, the mixing intensity provided by aeration was insufficient for complete mixing. Microscopic observations revealed that the proportion of very small algae (1-2 μm) gradually increased as mixing intensity diminished. The algae were still identified as Chlorella and Monodus, however.

As TSS concentration increased (i.e., algal growth entered the exponential growth phase), chitosan dosage requirements rapidly decreased from 38 mg chitosan/g TSS at day 2 to 15 mg chitosan/g TSS at day 5. As algal growth phase entered the stationary phases (days 6-8), DR_{50} reached an initial minimum (15 mg chitosan/g TSS) and then slightly increased to 18 mg chitosan/g TSS. Algal growth entered another exponential phase after day 8. The corresponding DR_{50} decreased slightly again. The minimum DR_{50} (11 mg chitosan/g TSS) was observed on day 13, when algal growth was in the late exponential phase. This value was 3.3 times less than the initial value.

These results indicate that flocculation of microalgae with chitosan is influenced by the growth phase of algae, with optimal conditions for flocculation existing in the late exponential growth phase. This is in agreement with the results of several investigators (Tenney et al., 1969; Avnimelech et al., 1982; Lavoie and de la Noue, 1983; Lavoie et al., 1984). This result might be attributed to the accumulation of biopolymer on algal

surfaces. Comparison of temporal variation of DR_{50} and the ratio of ash-free biopolymer to TSS (Figure 5-1) indicates that the least amount of flocculant is required when the ratio of ash-free polymer to TSS is at a minimum. While the algae remained in the stationary phase between days 6 and 8, the ratio of ash-free biopolymer to TSS was about 0.24. As algae entered the second exponential phase (days 9-14), this value fell to a minimum of 0.06 at day 12. Pavoni et al. (1971) found that the ratio of extracellular biopolymer to TSS reached a minimum during the late exponential growth phase of algae.

Two kinds of biopolymers which are visually discernible have been extracted from algal cultures in laboratory and field experiments. These are referred to as the "white flour" type and "yellow gum" type. Extracellular biopolymer extracted in this experiment was white colored and not sticky. Previous results (chapter 3) showed that this kind of biopolymer did not enhance algal bioflocculation. It was also observed in the field trials (chapter 4) that accumulation of this kind of biopolymer on algal surfaces inhibits chitosan flocculation. Tenney et al. (1969) postulated that accumulation of biopolymer at the cell surface in advanced growth phases enhanced chemical flocculation. This may apply only to exopolymers having characteristics like those of the yellow gum type found in this research.

Another observation was that DR_{50} and the ratio of biopolymer to TSS after day 14 both sharply increased as mixing intensity diminished. Excess biopolymers which accumulated on algal surfaces act as a protective colloid, covering active binding

sites and thus inhibiting chemical flocculation. Biopolymer accumulations could possibly be reduced by mixing, if the biopolymers were loosely bound to the cell surfaces.

5.3.2 Effect of mixing in the culture vessel

The temporal variation of algae removal efficiency, total suspended solids and chitosan dosage requirements for batch cultures under different mixing regimes is shown in Figure 5-2. Total suspended soilds (TSS) of the mixed cultures increased slightly whereas that of the unmixed culture decreased. Algal flocs (1-3 mm) were observed in the mixed cultures. Removal efficiencies by gravity settling at day 5 were 39% in the culture medium mixed at 40 rev/min and 28% in the medium mixed at 20 rev/min. These flocs were easily broken at the higher mixing intensity created by the magnetic stirrer during pH adjustment for flocculation test. The DR_{50}s of the mixed cultures declined from 15 to 4-5 mg chitosan/g TSS. The $DR5_0$ of the unmixed culture increased to 67 mg chitosan/g TSS.

In experiment L-9B (Fig. 5-3), the DR_{50} of mixed culture was reduced by 30-37% after the first day. A similar result was observed in the field experiments F-6 and F-7 (see chapter 4), where a 26% reduction in DR_{50} was observed after one day of mixing. The DR_{50} of activated sludge seeded culture was reduced by 41% after one day of mixing (experiment F-7). In this experiment, the two mixing speeds (40 rev/min or 20 rev/min) gave similar results. This indicates that a G of 7 s^{-1} is sufficient to markedly reduce chitosan dosage requirements in culture medium dominated by Chlorella and Monodus. The DR_{50} of unmixed culture

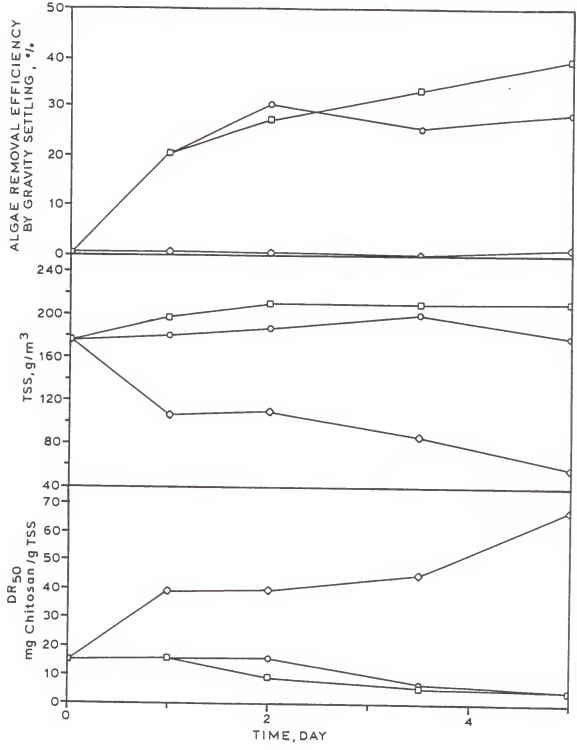

Figure 5-2. Temporal variation of algae removal efficiency, total suspended solids, and chitosan dosage requirements in batch cultures under different mixing regimes (Exp. L-8A). ◇ = un-mixed, ○ = 20 rev/min, □ = 40 rev/min

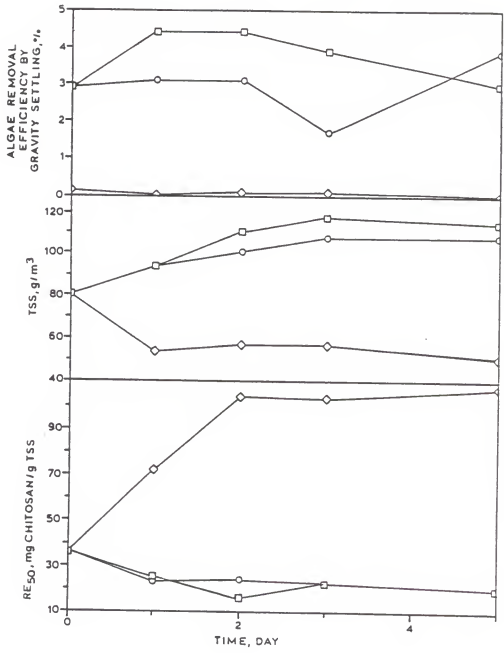

Figure 5-3. Temporal variation of algae removal efficiency, total suspended solids, and chitosan dosage requirements in batch cultures with different mixing regimes (Exp. L-9B). ◇ = un-mixed, ○ = 20 rev/min, □ = 40 rev/min

increased to 65 mg chitosan/g TSS after one day and to over 100 mg chitosan/g TSS after 5 days.

Variations of DR_{50} between mixed and unmixed cultures could be due to the accumulation of loose biopolymer and the change of conformation and orientation of loose biopolymers. As observed previously (section 5.3.1), the accumulation of loose biopolymer in unmixed culture may inhibit chitosan flocculation. Because the loose biopolymer is not firmly attached to cells, binding between chitosan and loose polymer would not enhance flocculation. When the culture is mixed, the conformation of accumulated biopolymers could be changed from a globular form to a finger-like form or even detached by the increased drag forces. Thus, the exposure of algal surface adsorptive sites would be increased. Increased binding between algal surface and chitosan should increase the effectiveness of chitosan.

CHAPTER 6
EFFECT OF MIXING, BIOPOLYMER AND CULTURE MEDIUM ON ALGAE FLOCCULATION WITH CHITOSAN

6.1 Introduction

Previous results (chapter 4 and 5) have shown that mixing in the culture vessel can reduce chitosan dose requirements considerably. This could be due to differences in conformation and production of biopolymer. Lavoie and de la Noue (1983) reported that algal cultures grown at 25° C required more chitosan to achieve the same degree of algal removal as culture grown at 5° C. They postulated that this was due to differences in biopolymer production. Tenney et al. (1969) hypothesized that excess accumulation of extracellular material on algal surfaces could inhibit flocculation by acting as a protective colloid.

Polysaccharides have been shown to reduce the sedimentation rate of Escherichia coli in the presence of aluminum sulfate and polyacrylamides (Roberts et al., 1974). Thus, in wastewater treatment processes it is necessary to adjust the amount of polyacrylamide in order to compensate for changes in the level of polysaccharides. Bitton et al. (1976) found that the flocculation of Klebsiella aerogenes $K_{54}A_3$ with clay mineral (montmorillonite) was dependent on the amount of capsular polysaccharide. The extent of cellular flocculation decreased when the polysaccharide content increased. Narkis and Rebhun (1983) examined the effect

of the content of organic matter (e.g., humic and fulvic acids) in tricking filter effluents on flocculant dose requirements. They found that cationic flocculant reacted preferentially with humics before flocculating suspended solids.

6.2 Materials and methods

6.2.1 Flocculation test

All experiments in this study were carried out using the micro-jar test procedure were described in Appendix A. The paddles which had a height of 1.5 cm and length of 1.75 cm were used. Culture was analyzed for its TSS concentration and then was adjusted to pH 6.5 with 8 N H_2SO_4.

6.2.2 Preparation of chitosan solution

Chitosan used for this study was made from snow crab shells (Protan lot # 123-121-03). 0.1% chitosan solution was prepared by diluting with distilled water. Details of preparation were described in section 4.3.2.

6.2.3 Extraction of biopolymer

Separation of soluble biopolymer was conducted by filtration. Culture was passed through a membrane filter (pore size of 0.2 μm, Gelman Sciences lot # 4065054). A 30 mL of filtrate was combined with 60 mL absolute ethanol in a vial and stored at 4° C for 24 h to allow precipitation of fibrous materials. Total extracellular biopolymer was extracted using the centrifugation technique described by Ueda (1963). Details of the extraction procedure were given in section 2.3.7. Glucose and protein concentrations in the biopolymer were determined according to methods described in section 2.3.8.

6.2.4 Experimental procedures

6.2.4.1 Effect of mixing in the culture vessel and resuspension of algae in distilled water: Experiment L-10

A sample of culture medium dominated by Chlorella and Monodus was taken from the high-rate pond. An initial flocculation curve was developed using this medium. Two-litre aliquots of the medium were transfered to macro gator jars. One jar was mixed at 40 rev/min ($G = 34\ s^{-1}$) by a standard jar test apparatus (Phipps and Bird). Another jar was not mixed. Both jars were illuminated by a 40 W fluorescent light placed 5 cm from the jars. After 24 h, flocculation curves were developed using 700 mL from each jar. The remaining volume was centrifuged at 10,000 x g for 20 min at 4° C. After carefully decanting the centrate, the algal pellet was resuspended with distilled water, and centrifuged again. The washed pellet was resuspended with a volume of distilled water equal to that of the first centrate decanted. A third pair of flocculation curves were developed using the washed cultures. Contrary to mixed culture, settled algae in the unmixed culture were excluded from the second and third flocculation tests. Soluble and total extracellular biopolymers of each culture were measured before the start of mixing and after 24 h.

6.2.4.2 Effect of added culture medium and added biopolymer: Experiments L-11A and L-11B

At the start of experiment L-11A, 3.6 L of culture medium from the high-rate pond was equally transferred into two 2 L beakers and placed under the light fixture described in section 3.3.1. Cultures were not mixed. After 2 days, the supernatant of culture medium was centrifuged at 10,000 x g for 20 min at 4° C.

The centrate was carefully collected to avoid contamination of algae or other suspended solids. The algal pellets were washed as described in section 6.2.4.1. One pellet was resuspended in distilled water and the others in mixtures of centrate and distilled water. The centrate:distilled water ratios of the mixtures were 15:85 and 50:50, respectively. Flocculation curves were developed using the control, algae resuspended in distilled water, and algae resuspended in mixtures of distilled water and centrate. The remaining centrate was extracted for biopolymers. One part of centrate was combined with two parts of absolute ethanol and stored at 4° C for 24 h. Fibrous materials were separated by centrifugation at 10,000 x g for 10 min at 4° C. Supernatant of the water and ethanol mixture was discarded. The remaining pellet (whitish material) was dissolved in distilled water. The volume of added distilled water was equal to that of centrate which was used for biopolymer extraction. Concentrations of ash-free biopolymer and ash were 303 and 163 g/m^3, respectively.

To begin experiment L-11B, fresh culture medium from C-4 was centrifuged at 10,000 x g for 20 min at 4° C. After discarding the centrate, the algal pellet was washed and resuspended in distilled water. Various amounts of extracted biopolymer from experiment L-11A were added to aliquots of resuspended culture to give final concentrations of ash-free biopolymer of 23, 45, and 76 g/m^3, respectively. These were shaken vigorously for 30 s. Flocculation curves were then developed.

6.3 Results and discussion

6.3.1 Effect of mixing in the culture vessel and culture medium replacement with distilled water

Figure 6-1 shows the effect of mixing in the culture vessel on chitosan dosage requirements. Relative to the original C-4 medium, the DR_{50} was reduced (by 22%) after 24 hours of mixing in the culture vessel whereas it was increased (by 37%) in the culture allowed to stand 24 hours without mixing. Soluble or loose biopolymer concentrations of each culture (sum of glucose and protein concentrations) differed slightly between the cultures, but the contents of total ash-free biopolymer were similar (Table 6-1). TSS concentrations of the cultures differed considerably from each other due to sedimentation and algal growth. Unit biopolymer concentrations (e.g., soluble or loose biopolymer and total ash-free biopolymer concentration per unit biomass) were likewise substantially different from each other. Those of the control, mixed and unmixed cultures were 0.11, 0.09, and 0.12 for soluble or loose biopolymer and 0.24, 0.21 and 0.25 for total ash-free biopolymer, respectively. This result indicates that the extent of the algal flocculation with chitosan decreased as the loose or soluble polymer content increased.

Removal of extracelluar biopolymers by centrifugation and reduction of ionic strength by replacement of culture medium with distilled water reduced the DR_{50} of mixed and unmixed culture by 66% and 80%, respectively (Fig. 6-1). The DR_{50}s were identical for the mixed and unmixed cultures after biopolymer removal and supernatant replacement.

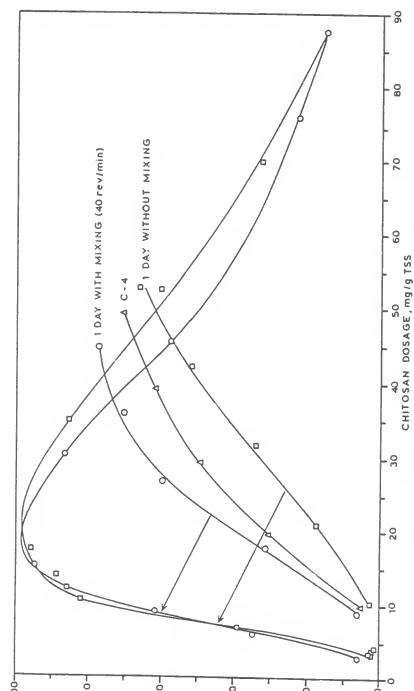

Figure 6-1. Effect of mixing in the culture vessel and culture medium replacement with distilled water on chitosan flocculation curves. Polymer extracted culture; □ = un-mixed, ○ = mixed

Table 6-1. Total suspended solids, biopolymer concentration and biopolymer composition (experiment L-10).

	Control culture (C-4)	Mixed culture for 24 h	Unmixed culture for 24 h
TSS (g/m^3)	546	600	510
Soluble or loose polymer (g/m^3)	57.9	56.9	61.8
Glucose	33.7	33.3	35.9
Protein	24.2	23.6	25.9
Total ash free polymer (g/m^3)	128.7	128.5	128.5
Glucose	77.8	77.9	78.1
Protein	50.9	50.7	50.4
Soluble or loose polymer per TSS	0.106	0.095	0.121
Total ash free polymer per TSS	0.236	0.214	0.252

Total ash free polymer and soluble or loose polymer concentrations were estimated by the sum of glucose and protein concentrations.

6.3.2 Effect of culture medium composition and added biopolymer

Figure 6-2 shows the effect of culture medium composition on algae flocculation curves with chitosan. Addition of centrate to algae resuspended in distilled water increased chitosan dosage requirements. For 50% of algal removal, dosages of chitosan for culture having no centrate, 15%, and 50% of centrate, and for control culture which was not polymer extracted, were 5, 12, 25, and 32 mg chitosan/g TSS, respectively. A linear relationship was found between fraction of centrate and dose requirement for 50% algal removal. This is in good agreement with Shelef et al. (1984). They observed that with increase of ionic strength from 0.1 to 0.7, alum optimal dosages for algal flocculation increased linearly. The flocculant requirement of the control for 50% algal removal (32 mg chitosan/g TSS) was considerably less than the expected value of 45 mg chitosan/g TSS, which was estimated considering the difference of ionic strength. This indicates that besides the effect of ionic strength, a considerable amount of biopolymer is present in the centrate. These biopolymers compete with suspended matter (e.g., algae) for flocculant.

Figure 6-3 shows the effect of added biopolymer on algae flocculation with chitosan. Flocculation was impaired by addition of extracted biopolymers. The extent of inhibition by biopolymer was slightly less than expected because the dose required for 50% removal was not much different from that seen in Figure 6-2. In terms of biopolymer concentration, 45 mg/L of added biopolymer in Figure 6-3 was equivalent to culture medium containing 15% centrate by volume. The DR_{50} in this case was 7.5 mg chitosan/g

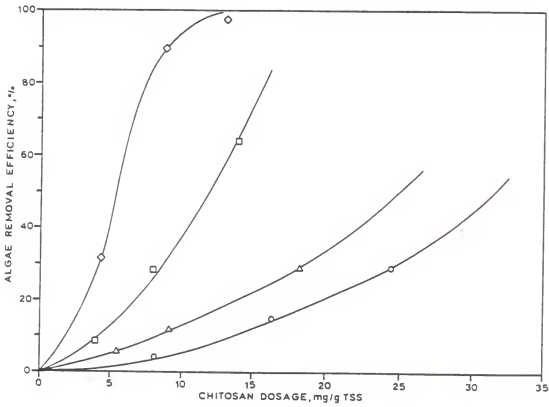

Figure 6-2. Effect of culture medium composition on chitosan flocculation curves. ○ = un-mixed algal culture (without polymer extraction), △ = polymer extracted algae resuspended with 50% of centrifuged supernatant and 50% of distilled water, □ = polymer extracted algae resuspended with 15% of supernatant and 85% distilled water, ◇ = polymer extracted algae resuspended with distilled water alone.

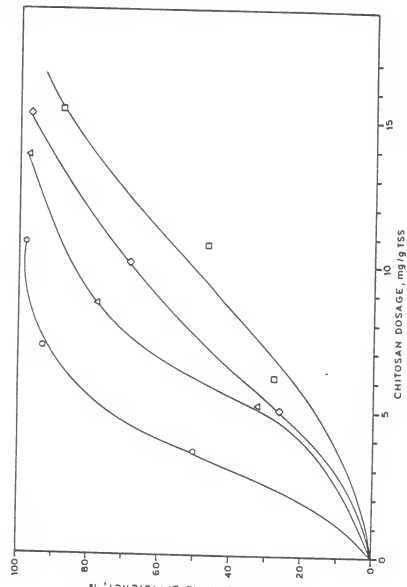

Figure 6-3. Effect of added biopolymer on chitosan flocculation curves. Dose of exopolymer extracted from algal culture; ○ = 0 g/m^3, △ = 23 g/m^3, ◇ = 46 g/m^3, □ = 76 g/m^3.

TSS, whereas that of equivalent culture in Figure 6-2 was 12 mg chitosan/g TSS. This difference could be attributed to ionic strength, variation of polymer structure and difference of culture characteristics. Cheng et al. (1975) found that extracted biopolymer has a lower adsorptive capacity for heavy metals than in situ biopolymers. They postulated that polymer extracted could be altered in structure so that its characteristics might be changed significantly. The change of characteristics of extracted bioplymer could be expected in this experiment because precipitation of extracted biopolymer was achieved in the ethanol solution. Comparing Figure 6-2 to Figure 6-3, flocculation curves of resuspended cultures with distilled water alone were slightly different. DR_{50} in Figure 6-2 was 5 mg chitosan/g TSS, whereas that in Figure 6-3 was 4 mg chitosan/g TSS. This indicates that there could be difference in flocculation due to culture characteristics. Algal species composition and degree of biopolymer extraction might be different between these experiments.

PHASE III.

AUTOFLOTATION

CHAPTER 7
EFFECT OF PHYSICOCHEMICAL AND BIOLOGICAL VARIABLES ON ALGAL AUTOFLOTATION

7.1 Introduction

The transport of particulates in liquid to the surface via small gas bubbles is referred to as flotation. This process is generally considered more advantageous than sedimentation for algae harvesting because it can be carried out at relatively high overflow rates while still attaining favorable separation efficiencies and a highly concentrated algal sludge. The extent of algal removal is determined, in part, by the available bubble surface area to which cells can adsorb. Very small bubbles are thus desirable. Mechanical systems, such as pressurized aeration (van Vuuren et al., 1965; Bare et al., 1975; Shannon and Buisson, 1980), CO_2 injection (Conway et al., 1981), and electrolysis (Sandbank et al., 1974; Kumar et al., 1981) have generally been used to produce minute gas bubbles. These systems are generally complex and energy intensive, however. The supersaturated dissolved oxygen which originates from the photosynthetic activity of algae in high-rate ponds has received some attention as an alternative source of flotation gas. Van Vuuren and van Duuran (1965) first observed flotation of algae by photosynthetic oxygen, a process later termed "autoflotation" (Anon., 1972). Several researchers have applied autoflotation to harvest algae from

municipal pond systems (van Vuuren and van Duuren, 1965; van Vuuren et al., 1965; Cillie et al., 1966; Parker et al., 1973) and high-rate ponds (Arbelaez et al., 1983; Koopman and Lincoln, 1983).

The purpose of this study was to determine the effects of several environmental and management variables on the autoflotation process. These variables included dissolved oxygen, mixing and turbulence, flocculant characteristics, and algal species and physiological state. The performance parameters monitored were algal rise rate, separation efficiency, and algal solids concentration.

7.2 Literature review

7.2.1 Theoretical considerations

Autoflotation involves the rapid transfer of oxygen from the liquid to gaseous phase in a flocculent or flocculating algal suspension. The rate of oxygen transfer can be described by Fick's first law expressed in concentration units.

$$\frac{dC}{dt} = K_L a \, (C_s - C) \qquad (7\text{-}1)$$

where

$\frac{dC}{dt}$ = rate of change in concentration of dissolved oxygen $(g/m^3 \cdot s)$,

$K_L a$ = overall mass transfer coefficient (s^{-1}),

C_s = saturation concentration for dissolved oxygen (g/m^3),

C = actual concentration of dissolved oxygen (g/m^3).

The saturation DO concentration can be calculated using Henry's Law. This value is primarily a function of temperature, but is also affected by salinity and pressure. Actual DO concentrations in oxidation ponds commonly exceed saturation during periods of active algal photosynthesis (Kormanik and Cravens, 1979; Kaza, 1971; Friedman et al., 1977; van Vuuren et al., 1980).

Three gas-liquid transfer models are commonly used: two-film, penetration and surface renewal. The two-film theory (Fig. 7-1) is the oldest and simplest and is by far the most widely used model for descibing gas transfer in water and wastewater (Schroeder, 1977). According to the two-film theory, gas molecules must penetrate both gas and liquid films in order for transfer to occur. The films' thickness and character significantly affect gas transfer rate. In the case of oxygen transfer, the liquid-film resistance is considerably greater than the gas-film resistance and hence controls the oxygen-transfer rate across the interface. The overall mass transfer coefficient, K_La, reflects the liquid film resistance as well as other characteristics of the system. Physicochemical factors affecting the value of K_La include: (1) temperature, (2) gas-liquid contact time, (3) turbulence, (4) the presence of surfactant, and (5) the ratio of interface area/liquid volume.

According to equation (7-1), the rate of oxygen transfer under supersaturated conditions is directly related to K_La and the excess of actual DO over the saturation concentration. The

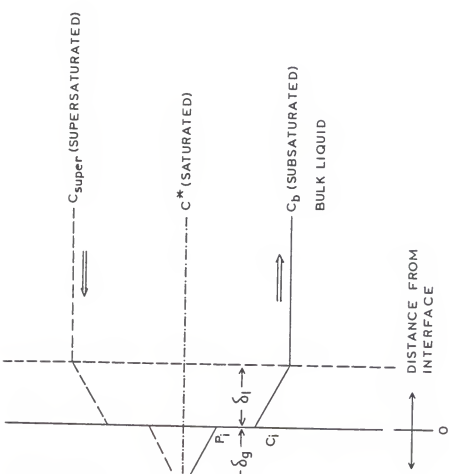

Figure 7-1. Schematic diagram of two-film gas transfer model.

negative sign on the result indicates that transfer is from the liquid to the gaseous phase.

7.2.2 Flotation mechanisms

There are three distinct mechanisms which account for the flotation of gas bubble-algal floc aggregates (Vrablik, 1959; Packham and Richards, 1972; Kitchener and Gochin, 1981; Gochin and Solari, 1983):

(1) Entrapment of pre-formed bubbles within condensing floc structures, either during floc formation or due to floc breakage and reformation within the flotation tank or as a result of the "filtering out" of rising bubbles by settling flocs.

(2) Growth of bubbles from nuclei on solids within floc networks.

(3) Attachment of flocs to bubbles on collision.

Mechanism (1) may be important in situations where the rate of coagulation is high, but is not significant in dilute systems. Mechanism (2) was observed when bubble-free supersaturated water was injected underneath a flocculent suspension. After an induction period (several minutes), bubbles appeared in the flocs (Gochin and Solari, 1983). Mechanism (3) is significant in the dissolved air flotation process (Kitchener and Gochin, 1981; Gochin and Solari, 1983). In this case, the attraction of oppositely charged particles and bubbles is feasible as long as turbulence brings them close enough to allow electrical double layers to interact (Bleier et al., 1976). Also, the particles' hydrophobicity was found to be critical factor controlling flotation response (Gochin and Solari, 1983). Flotation in this

case is achieved in a relatively short time, without an induction period.

7.2.3 Experience with the autoflotation process

Van Vuuren and van Duuren (1965) first observed autoflotation in laboratory when algae in the maturation-pond effluent was flocculated with aluminum sulfate. During pilot-plant tests, they achieved 80% of algae removal efficiency at a basin flow rate of 0.67 m/h. Recovered algal slurries averaged 1.4% solids by weight.

Cilie et al. (1966) found that the autoflotation of algal floc were related to the rate of mixing, i.e., the turblent conditions which pertained. Complete flotation was observed when the algal culture was stirred gently for only a few seconds. Prolonged stirring culture (2-3 min) hindered flotation. They reported that a minimum DO of 14 g/m^3 was needed for flotation with photosynthetic oxygen.

Van Vuuren et al. (1965) applied autoflotation to facultative pond effluent. 70% algae removal efficiency was obtained at a basin over flow rates of 4.4-7.6 m/h. They found an influence of synthetic detergents (ABS) on the flotation process. Without ABS, the efficient flocculation and flotation was hindered. They suggested that the use of polyelectrolytes along with flocculants could accelerate the flocculation and improve the flotation.

In 1972, Parker et al. (1973) evaluated autoflotation at a tertiary pond system receiving both municipal and canning wastes. They found that DO levels exceeding 13-15 g/m^3 were required for successful performance.

Arbelaez et al. (1983) reported the existence of a weakly positive correlation between algal rise rate and ΔDO. Minimum DO required for flotation was reported to be 3-5 mg/L greater than typical saturation concentrations. Above the this level for flotation, the performance of autoflotation was insensitive to DO concentration. They indicated that the duration and intensity of initial mixing between flocculant and algal suspension greatly affects the efficiency of the autoflotation process. Long contact times and low mixing intensity were found to be detrimental to flotation intensity (i.e., floc rise rate) and stable float formation.

Koopman and Lincoln (1983) applied autoflotation to high-rate pond effluents. They achieved 80-90% algae removals at overflow rates in the flotation basin of up to 2 m/h with algal float concentrations averaging more than 6% solids.

7.3 Materials and methods

7.3.1 Algal species

Chlorella and Monodus were dominant while most of the autoflotation experiments were conducted (13-30 June and 26-31 July, 1985). Flagellates such as Euglena and Chlamydomonas sometimes appeared on the surface of pond as a thin film. During late September 1985, minute blue green algae identified as Synechocystis were prevalent comprising over 99% of the algal biovolume.

7.3.2 Test apparatus

Autoflotation was tested at both the pilot and field scales. Each test apparatus consisted of the following elements: inlet and

pump, tubular reactor, and flotation cylinder or tank. The surface intake, located in the terminal high-rate pond (C-4), consisted of a galvanized steel funnel attached via a sliding connection to a riser of 3.2 cm I.D. polyvinyl chloride (PVC) plastic pipe (Fig. 7-2). Buoyancy was provided by a circular polystyrene float attached to the funnel so as to maintain a funnel submergence of 2 cm. This submergence was self-controlled through a vertical distance of 10 cm, the range of travel of the sliding connection, which was generally more than enough to accomodate the water level drop in pond C-4 during flotation trials. Two intersecting baffles were placed vertically within the funnel to prevent vortexing. After withdrawal, culture media flowed a distance of 4 m through 5.4 cm I.D. PVC pipe to the suction port of a 1.9 kW centrifugal pump.

In the pilot scale apparatus, three different configurations of 5.4 cm I.D. PVC pipe were used to connect the pump with the flotation cylinder (Fig. 7-3). The total lengths of configurations including the inlet portion already described, were 7.1, 12.9 and 25.8 m, respectively. Mixing intensity (as measured by mean velocity gradient; G) was varied by adjusting flow rate. Total retention time in the piping system (t_d) was a function of flow rate and reactor length. Flocculant contact time (t_c) was varied by changing the distance between the flocculant injection point and the flotation cylinder. t_c was also influenced by the flow rate. The DO of the high-rate pond effluent was monitored in the tubular reactor immediately down stream of the pump (Fig. 7-3). Flocculant solutions were injected by peristalic pump into

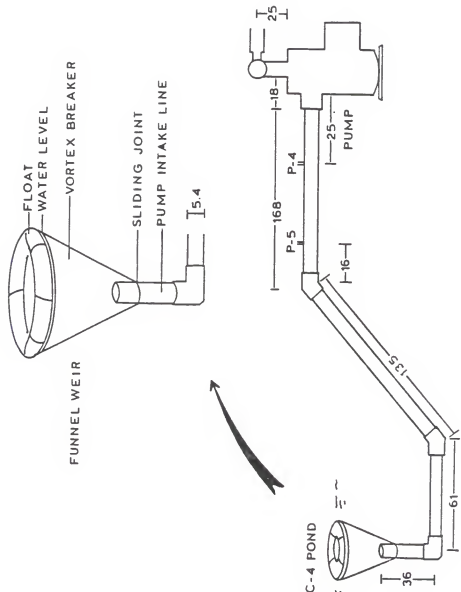

Figure 7-2. Floating intake and inlet portion of autoflotation system. (All dimensions are given in cm.)

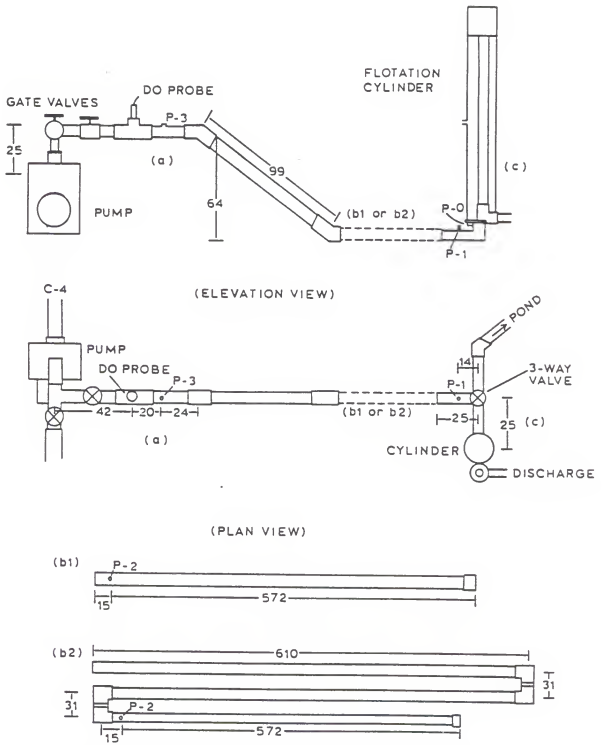

Figure 7-3. Outlet portion of pilot scale autoflotation system. (All dimension are given in cm.)

the main flow by a diffuser (Fig. 7-4 top) that could be inserted at various positions along the reactor. When chitosan solution was used as flocculant, pH was adjusted to 6.5 by injection of 8 N H_2SO_4 at position P-3, 20 cm downstream of the DO electrode, but prior to the flocculant injection points. The diffuser used to inject acid is shown in the bottom portion of Figure 7-4. A three-way valve placed at the end of the reactor directed flow to the autoflotation cylinder or to a bypass leading back to the high rate pond (Fig. 7-3).

The autoflotation cylinder was fabricated from a 1.3 m length of 8.6 cm I.D. acrylic plastic tubing with an effluent launder attached near the top to allow continuous flow-through (Fig. 7-5). The cylinder was shrouded partially by aluminium foil to limit illumination by sunlight. Flow entered the bottom of the cylinder via a long-sweep, 90 degree elbow. The liquid depth in the cylinder was 103 cm.

When autoflotation was tested at the field scale, 11 m of 7.6 cm I.D. PVC pipe was used to connect the pump to the autoflotation tank. The tank (Fig. 7-6) had a diameter of 3.6 m and side water depth of 0.6 m. Culture medium entered the tank tangentially at the bottom well, setting up a swirling motion. Acid (8 N H_2SO_4) and chitosan solution were injected via diffusers at points P-5 and P-4, respectively (Fig. 7-2). pH was maintained at about 6.4.

7.3.3 Flocculants

The flocculants used were alum and chitosan. Alum has been widely used as a primary flocculant for autoflotation (van Vuuren

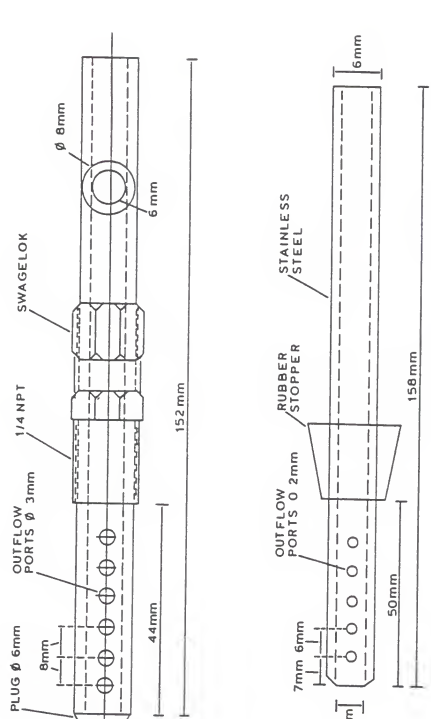

Figure 7-4. Diffusers used to inject flocculant (top) and acid (bottom).

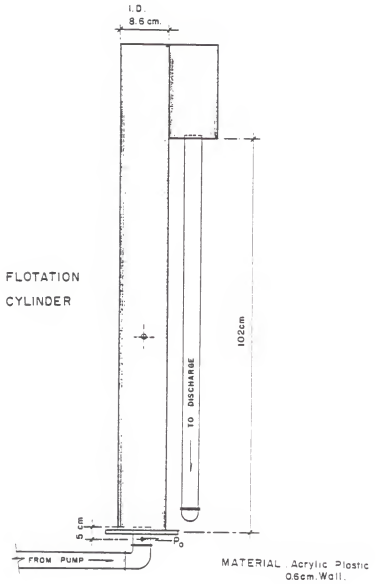

Figure 7-5. Autoflotation cylinder.

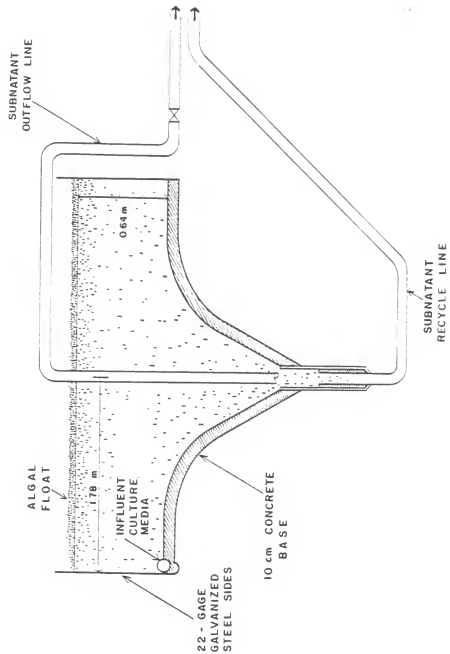

Figure 7-6. Autoflotation tank.

and van Duuren, 1965; van Vuuren et al., 1965; Cilie et al., 1966; Parker et al., 1973; Arbelaez et al., 1983; Koopman and Lincoln, 1983). It is easily and homogenously dispersed with relatively weak mixing intensity (Hudson, 1981). The alum stock solution (14% as $Al_2(SO_4)_3 \cdot 18H_2O$) was made up daily. The solution of alum was dosed into the algal suspension at the appropriate rate to provide a dosage of approximately 1.2 g alum/g TSS.

The chitosan used in this study was prepared from snow crab shells (Protan; lot no. 123-121-03). A 1.0% solution of chitosan was made up weekly in 1% acetic acid and stored at 4° C.

7.3.4 Determination of mean velocity gradient, total retention time, and flocculant contact time

7.3.4.1 Mean velocity gradient in pipes

Mean velocity gradient may be calculated according to the following equation (Fair et al., 1968):

$$G = (P/\mu C)^{1/2} \qquad (7\text{-}2)$$

where

P = power input (W),

C = fluid volume (m^3),

μ = dynamic viscosity ($N \cdot s/m^2$), and

G = mean velocity gradient (s^{-1}).

Power input is the energy dissipated in the reactor, which for a pipe is given by:

$$P = pgQh_{Lp} \qquad (7\text{-}3)$$

where

p = fluid density (kg/m^3),

g = gravity constant (m/s^2),

h_{Lp} = head loss (m) in pipe, and

Q = flow rate (m^3/s).

To calculate head loss in the pipe due to frictional resistance, Arbelaez et al. (1983) used the Darcy-Weisbach equation:

$$h_{Lp} = fLv^2/D2g \qquad (7\text{-}4)$$

where

f = friction factor (dimensionless),

L = length of reactor (m),

v = mean velocity (m/s), and

D = pipe diameter (m).

They combined equations 7-2, 7-3 and 7-4 to yield:

$$G = (1.032pfQ^3/uD^7)^{1/2} \qquad (7\text{-}5)$$

Equation 7-5 was used with f = 0.011 (Plastic Piping Systems, Inc., 1980) to calculate mean velocity gradients in the pipes of the test apparatus.

7.3.4.2 Mean velocity gradient in the pipe fittings

The head loss through the gate valve, elbows and 3-way valve was calculated according to the following equation:

$$h_{LM} = Mv^2/2g \qquad (7\text{-}6)$$

where

h_{LM} = minor head loss

M = minor loss coefficient.

Values of M for the gate valve were determined from Figures 7-7 and 7-8. Values of 0.255 and 0.25 (Benefield et al., 1984) were used for the 90° and 45° elbows, respectively. The 3-way valve was treated as a 90° elbow. The volumes of water in the gate valve, 90° elbows and 45° elbows were 0.24 L, 0.36 L and 0.28

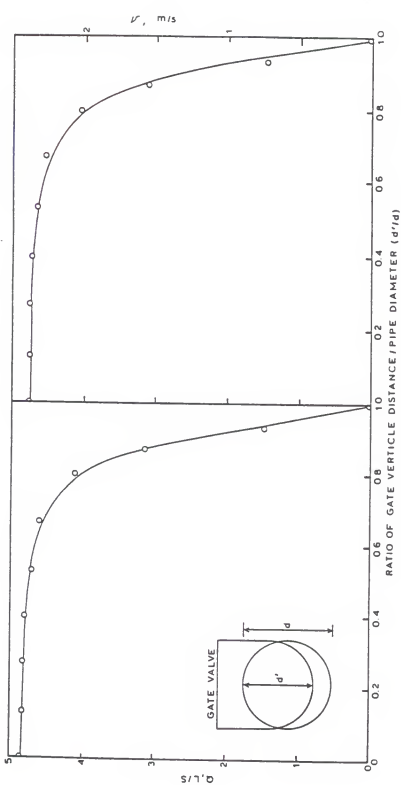

Figure 7-7. Relationship of gate valve position to flow rate and velocity.

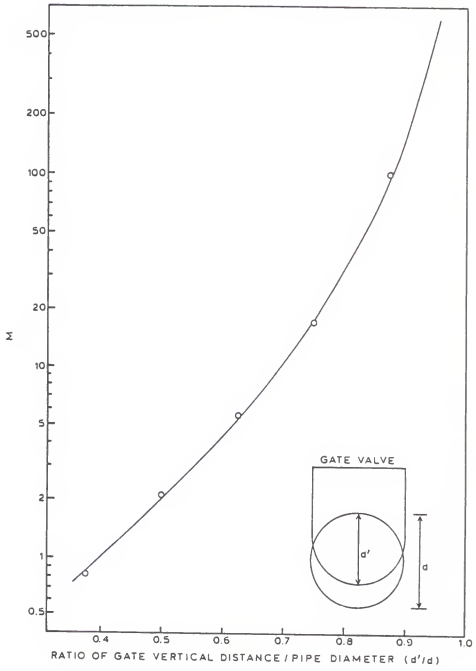

Figure 7-8. Relationship between gate valve position and minor loss coefficient M. (After Merriman 1929)

L, respectively. Velocity gradients in the gate valve and elbows were calculated by combining equations 7-2, 7-3 and 7-6, as shown below:

$$G = (0.203p\dot{M}Q^3/uCD^2)^{1/2} \qquad (7\text{-}7)$$

7.3.4.3 Mean velocity gradient in the pump

Pump head was calculated according to:

$$H_p = H_s + \Sigma h_{LP} + \Sigma h_{LM} \qquad (7\text{-}8)$$

where

H_p = pump head (m),

H_s = static head (1.5 m),

The pump head-capacity curve estimated in this manner is shown in Figure 7-9 (lower curve). Also shown in this figure is the manufacturer's curve (upper curve). The discrepancy between the two curves can be attributed to the age, condition and application of the pump. The unit is five years old and operates outdoors. The resulting wear and corrosion of the impeller, casing, bearings and other parts have certainly reduced the head produced by the pump. Also, the pond water commonly contains grass and other debris which fall in from the sides. This material tends to collect on the impeller, reducing pumping efficiency.

Mean velocity gradient in the pump was calculated by combining equations (7-2), (7-3) and (7-8), as shown below:

$$G = (pgQH_p/\mu C)^{1/2} \qquad (7\text{-}9)$$

The liquid volume of the pump was 6.55 L.

7.3.4.4 Total retention time and overall mean velocity gradient

Retention times in the pipes, pipe fittings and pump were calculated according to:

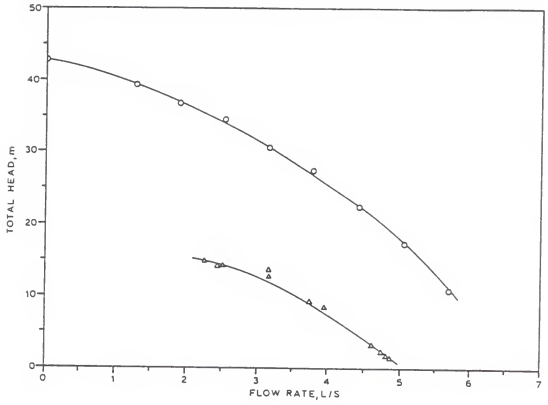

Figure 7-9. Head capacity curves for the centrifugal pump. ○ = manufacturer's curve (A.O. Smith Corp., DS2HG-53), △ = estimated curve.

$$t_{di} = V/Q \qquad (7\text{-}10)$$

where

V = liquid volume (m^3).

The overall mean velocity gradient (G_{avg}) was calculated according to:

$$G_{avg} = \Sigma G_i \cdot t_{di}/\Sigma t_{di} \qquad (7\text{-}11)$$

where

G_i = mean velocity gradient in each pipe or pipe fitting (s^{-1})

t_{di} = liquid retention time in each pipe or pipe fitting

Σt_{di} = total retention time.

7.3.4.5 Flocculant contact time

Flocculant contact time (t_c) was calculated from:

$$t_c = L_d/v \qquad (7\text{-}12)$$

where

L_d = length of pipe from the flocculant injection point to flotation cylinder (including all fittings).

7.3.5 Pilot scale test procedure

7.3.5.1 Summary of experiments

The schedule of experimental trials is given Table 7-1. Culture characteristics (TSS, algal species) changed during the four month period over which trials were conducted, but were relatively constant during the short time interval (1-5 days) during which a particular variable was tested. Table 7-1 also gives the experimental conditions (e.g., pH, flocculant dosage, total pipe length) employed while testing each variable and lists the parameters monitored.

Table 7-1. Description of autoflotation trials conducted with pilot scale apparatus.

Date	Exp. No.	Culture TSS (g/m^3)	Dominant algae [2]	Flocculant Type	Flocculant Dosage (g/g TSS)	Total pipe length (m)	Pipe flow velocity (m/s)	Dose point	Parameters monitored
June 13-18	F-8 F-9	1053	C/M	Alum	1.2	7.1 12.9 25.8	1.09	P-0	Rise rate (Vr_1 & Vr_2) DO, pH, TSS
19, 25-26	F-9	980	C/M	Alum	1.2	7.1 12.9 12.9	0.66 0.69 1.84	P-0	Vr_1 & Vr_2 DO, pH TSS
21-25	F-13	940	C/M	Alum	1.2	12.9	1.84	P-0	Vr_1 & Vr_2 DO, pH, TSS
27-29	F-9	1010	C/M	Chitosan	0.05	7.1	0.19 0.44 0.66 1.57	P-0	Vr_3, DO, pH TSS
30	F-8 F-9	1003	C/M	Chitosan	0.05	7.1 12.9 25.8	1.20	P-0	Vr_3, DO, pH TSS
July 27	F-8	840	C/M	Alum Chitosan	1.2 0.05	12.9	1.09 1.20	P-0	Vr_2 & Vr_3 TS, ash content in algal mats Thickness of algal mats
28-30	F-10 F-12	897	C/M	Alum Chitosan	1.2 0.05	12.9	1.05 0.87	P-0 P-1 P-2	Removal efficiency (%) Vr_1 & Vr_2, Vr_3 DO, pH, TSS

Table 7-1. continued

Date	Exp. No.	Culture TSS (g/m^3)	Dominant algae[2]	Flocculant Type	Flocculant Dosage (g/g TSS)	Total pipe length (m)	Pipe flow velocity (m/s)	Dose point	Parameters monitored
July 31	F-11 F-12	880	C/M	Chitosan	0.016 0.027 0.036 0.043 0.059	12.9	0.98	P-2	Removal efficiency (%) TSS, DO, pH
September 25	F-12	420	Sy	Alum	2.0	12.9	0.42	P-0	Vr_2, TSS, DO pH
26	F-11	410	Sy	Alum,	0.62 0.86 1.25 1.73 2.39 2.84	12.9	0.42	P-0	Removal efficiency (%) TSS, DO, pH
27	F-12	400	Sy	Chitosan	0.08	12.9	0.42	P-0	Vr_3, TSS, DO pH
29-30	F-11	380	Sy	Chitosan[3]	0.021 0.038 0.069 0.095 0.119	12.9	0.42	P-2	Removal efficiency (%) TSS, DO, pH

[2]Abbreviations for algal genera:
C/M = Chlorella and Monodus
Sy = Synechosystis
[3]Parallel flocculation sedimentation tests were carried out.

7.3.5.2 General procedure

Autoflotation trials with the pilot scale apparatus were conducted on the afternoons of relatively clear days to coincide with peak DO levels in the high-rate pond. Prior to each trial (generally at about 9 AM local time) the pump was operated briefly in order to obtain a sample of C-4 culture medium, which was subsequently assayed for TSS. The flocculant injection rate was set based on the target dosage, flow rate, and culture medium TSS.

At the start of a typical trial, the pump was turned on and the 3-way valve turned to direct flow through the cylinder. Flow rate was adjusted to yield a preselected G value and the flocculant injection rate was set accordingly. If chitosan was used, the acid injection was varied until a stable pH of 6.5 was obtained. DO and temperature were recorded next. Finally, with the system operating smoothly, the 3-way valve was turned to direct flow back to the high-rate pond, leaving a stationary column of water in the cylinder. This procedure required about 2-3 minutes to complete. When the dose point was downstream of the 3-way valve, at P-0, it was essential to stop flocculant injection at the same instant that the 3-way valve was turned. This was accomplished by positioning a remote on-off switch at the valve which controlled both the flocculant and acid injection pumps.

DO concentrations ranging from 13 to 36 g/m^3 were obtained by mixing the high-rate pond for varying periods before or between trials. Mixing disrupted the thermal stratification and with it the surface layer of oxygen supersaturated medium. Target G and

t_d values were obtained by controlling flow rate and by using different total pipe lengths (Table 7-2).

Floating algal mats in the autoflotation cylinder were sampled after nearly complete (>90%) algal flotation had occurred. With alum this corresponded to a batch flotation period of 4 min, whereas with chitosan the required period was 3 min. The sample was scooped off the surface with a 100 mL glass beaker, being careful to exclude subnatant water. Subnatant samples were taken through the sampling tap (Fig. 7-5) after batch flotation times of 4 min with alum and 3 min with chitosan.

7.3.5.3 Normalization of rise rates

In order to account for the effect of DO on rise rate, tests were conducted over wide ranges of DO concentration and normalized rise rates were estimated for selected DO excess (ΔDO) levels. The normalization procedure consisted of calculating the best fit relationship between rise rate and DO by least squares linear regression. For example, consider the relationship depicted in Figure 7-10. In this test, the best fit line was given by:

$$Vr_1 = 4.450\ \Delta DO - 9.401 \quad (n=12;\ r=0.9890) \quad (7\text{-}13)$$

where

Vr_1 = algal rise rate expressed in m/h,

ΔDO = excess of actual DO concentration over the calculated saturation DO (g/m^3),

n = number of observations, and

r = correlation coefficient.

The value of Vr_1 estimated for a ΔDO of 10 mg/L is 35 m/h, as shown in Figure 7-10.

Table 7-2. Values of $\Sigma G_i \cdot t_{di}$, t_d and G_{avg} for the combinations of flow rate and total pipe length employed in pilot scale trials

Total pipe length (m)	Pipe flow velocity (m/s)	Flow rate (L/s)	t_d (s)	$\Sigma G_i \cdot t_{di}$	G_{avg} (s^{-1})
7.1	0.19	0.39	60.1	68214	1135
7.1	0.44	0.89	26.6	45779	1721
7.1	0.66	1.34	17.7	38214	2159
7.1	1.09	2.21	10.7	29853	2790
7.1	1.20	2.43	9.8	29527	3013
7.1	1.57	3.18	7.5	26580	3544
12.9	0.69	1.39	25.5	39398	1545
12.9	1.09	2.21	16.1	32329	2008
12.9	1.20	2.43	14.6	31492	2157
12.9	1.84	3.73	9.5	24453	2574
25.8	1.09	2.21	28.6	38152	1334
25.8	1.20	2.43	26.1	37767	1447

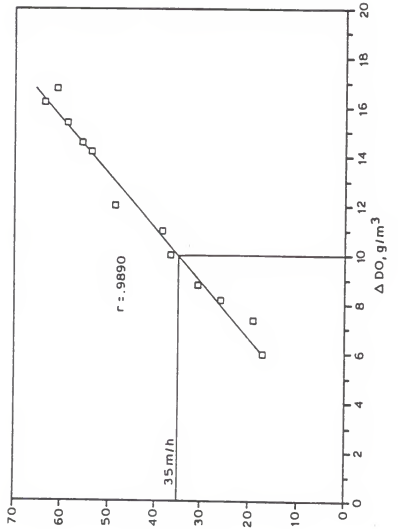

Figure 7-10. Normalization of algal rise rate. Test conditions: Flocculant dosage (Df) = 1.2 g alum/g TSS, Total pipe length (L_p) = 25.8 m, Pipe flow velocity (V_p) = 1.1 m/s, Dose point = P-0

7.3.5.4 Flocculation-sedimentation tests carried out in parallel with autoflotation trials

Flocculation-sedimentation tests were run in parallel with same flotation tests, to enable the solids contents of floated and settled algal slurries to be compared. A standard jar test apparatus (Phipps and Bird) was used for the flocculation-sedimentation tests. A sample taken from C-4 was adjusted to pH 6.5 using 8 N H_2SO_4 if chitosan was to be used. The flocculation-sedimentation tests were carried out according to the procedure described in Appendix A, except for the settling phase. After 15 min of slow mixing, the flocculent algal suspensions were carefully transfered to separatory funnels and a quiescent settling period of 30 min was allowed. At the end of the settling period, the settled algal slurries drawn off at the bottoms of the funnels and subsequently assayed for TS. Other flocculation-sedimentation tests were conducted using same batch of sample according to standard jar test procedure described in Appendix A. At the end of the settling period, 10 mL of supernatant was taken from each jar at 2 cm below the surface and analyzed for optical density.

7.3.6 Field scale test procedure

Culture medium dominated by Chlorella was adjusted to a pH of 6.4 by adding 8 N H_2SO_4 at P-5 (Fig. 7-2). Temperature and DO at the pond surface culture were 18° C and 26 g/m^3, respectively. Flow rate was 5.1 L/s. Chitosan was injected at P-4; dosage was 16 g chitosan/m^3 (26 mg chitosan/g TSS). The floating algal mat formed in the flotation tank during the harvest was allowed to build up for the full harvest period (105 min) with continuous

draining of subnatant. Samples of effluent were taken every 20 min and analyzed for removal efficiency. After the flotation test, algal slurry was transfered to a filtration-drying table. Total harvested algal biomass was weighed after 3 days on the table.

7.3.7 Analytical methods

DO was measured using an oxygen meter and polarographic electrode (YSI model 54). Electrode membranes were replaced daily and the DO meter was calibrated frequently (every 1-2 hours). The oxygen meter's range was expanded beyond the normal maximum of 20 g/m^3 by adding a resister to decrease its sensitivity by exactly 50%, allowing measurement of up to 40 g DO/m^3.

Algae removal efficiency was calculated according to the procedure described in section 2.3.4.

7.4 Results and discussion

7.4.1 Behaviour of floating algal flocs

When alum was used and the ΔDO (culture DO minus saturation DO) was relatively low (4-7 g/m^3), large flocs were formed (4-5 cm). Floc size decreased at higher ΔDOs, reaching a minimum of 1-5 mm at ΔDOs exceeding 27-29 g/m^3. A distinct interface formed between the rising algal flocs and the subnatant when rise rates were relatively low. With higher rise rates, the interface was diffuse in nature. The rising algal flocs eventually reached a zone of increasing concentration near the water column's surface, which slowed and eventually stopped their ascent. Plotted as a function of time, the position of the algal interface typically described a curve such as that shown in Figure 7-11. The initial

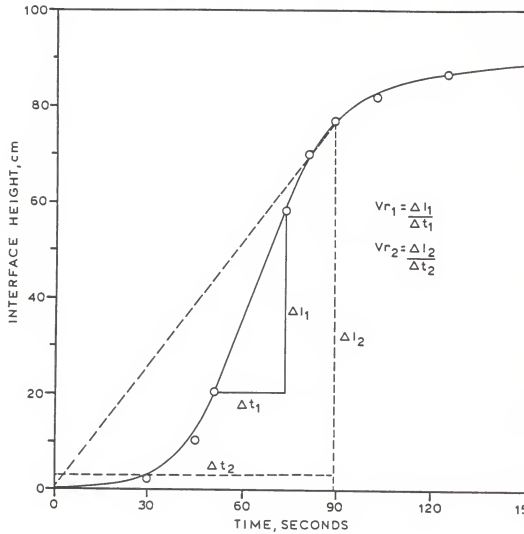

Figure 7-11. Typical plot of algal interface height versus time for a batch flotation test with alum. Test condition: D_f = 1.2 g alum/g TSS, L_p = 25.8 m, V_p = 1.1 m/s, Dose point = P-0.

region of the curve, representing the formation of an interface, is referred to as the flocculation phase. The region of the curve following flocculation is referred to as the initial flotation phase. The slope of this region (between interface heights of 20 cm and 58 cm) of the curve gives the initial rise rate (Vr_1) of the flocculated suspension. Subsequent regions of the curve represent transition and compression phases, respectively. Fast rising algal suspensions did not exhibit a discernable interface in the flotation zone, preventing the measurement of initial rise rate, Vr_1. With alum, the interface in such cases became visible as the transition region was entered. The overall rise rate, Vr_2, was calculated from the time required for the interface to rise to a height of 76 cm. This height was the minimum at which the interface could be consistently observed. As Figure 7-11 shows, rise rate Vr_2 is less than Vr_1 for the same data because it includes the flocculation phase and part of the transition phase, in addition to the initial flocculation phase.

With chitosan, an initial flocculation phase was not observed. Rising flocs were observed as soon as flow through the cylinder was stopped. An interface formed near the top of the cylinder as algal flocs accumulated at the water's surface. As algae continued to float to the surface, the interface moved downward, indicating that the accumulating algal flocs were in the compression phase. The thickness of accumulated algae in this case was approximately proportional to the mass of solids. When the algal concentration was in the range of 840-1010 g/m^3, a thickness of 5 cm was observed to contain over 90% of the algae

originally in the water column. When the algal concentration was in the range of 380-400 g/m^3, a thickness of 2.5 cm was observed to contain over 90% of the biomass. Rise rate, Vr_3, was calculated from the height of the interface (98 cm or 101.5 cm, respectively), divided by the time required for the algae to accumulate to a thickness of 5 cm if the culture TSS was 840-1010 g/m^3 or 2.5 cm if the culture TSS was 380-400 g/m^3.

7.4.2 Effect of dissolved oxygen on algal rise rate

Results obtained with alum are shown in Figures 7-12 and 7-13. Results obtained with chitosan are shown in Figures 7-14 and 7-15. Rise rates of algal suspensions flocculated with either chemical were strongly dependent on ΔDO.

When alum was used, the initial rise rate (Vr_1), which excludes the flocculation phase, did not increase beyond a ΔDO of 18 g/m^3. Vr_2, which includes the flocculation phase, continued increasing to a ΔDO of 26 g/m^3. This indicates that the length of the initial flocculation period is reduced as ΔDO increases. Results of this study show a greater sensitivity of algal rise rates to DO concentration than the results of Arbelaez et al. (1983).

When chitosan was used, rise rates (Vr_3) increased in proportion to the ΔDO, up to as much as 26 g/m^3.

7.4.3 Effect of piping system variables

Figures 7-12 and 7-14 indicated that rise rates were less at the greater piping lengths. This result implies that the performance of autoflotation would deteriorate as total pipe

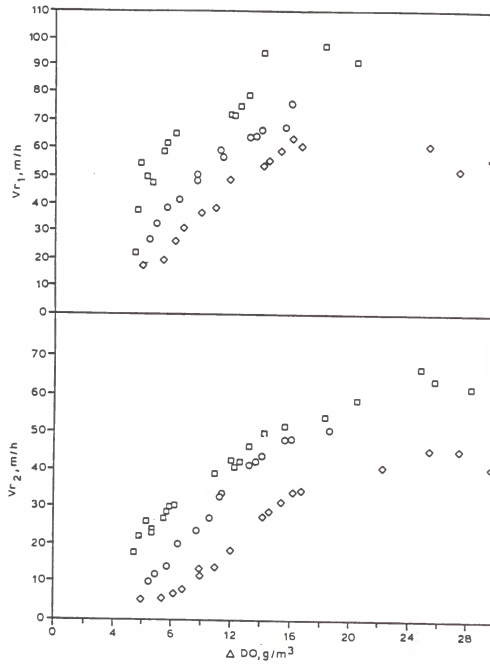

Figure 7-12. Effect of dissolved oxygen excess and total pipe length on rise rates of alum flocculated algae. Total pipe length: □ = 7.1 m, ○ = 12.9 m, ◇ = 25.8 m. Test conditions: D_f = 1.2 g alum/g TSS, V_p = 1.1 m/s, Dose point = P-0.

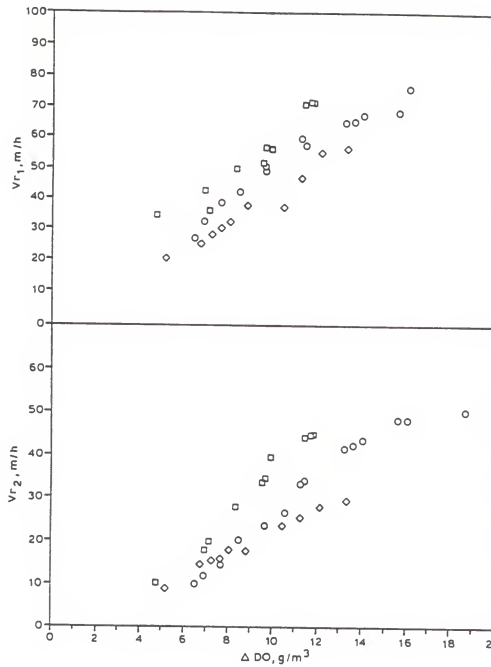

Figure 7-13. Effect of dissolved oxygen excess and pipe flow velocity on rise rates of alum flocculated algae. Pipe flow velocity: □ = 0.66 m/s, ○ = 1.09 m/s, ◇ = 1.84 m/s. Test conditions: D_f = 1.2 g alum/g TSS, L_p = 12.9 m, Dose point = P-0.

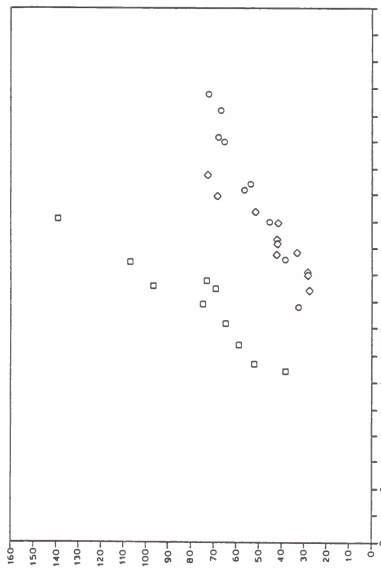

Figure 7-14. Effect of dissolved oxygen excess and total pipe length on rise rates of chitosan flocculated algae. Total pipe length: □ = 7.1 m, ○ = 12.9 m, ◇ = 25.8 m. Test conditions: D_f = 0.05 g chitosan/g TSS, V_p = 1.2 m/s, Dose point = P-0.

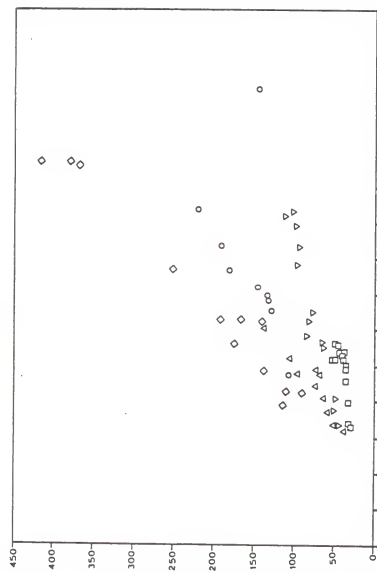

Figure 7-15. Effect of dissolved oxygen excess and pipe flow velocity on rise rates of chitosan flocculated algae. Pipe flow velocity: □ = 0.19 m/s, ○ = 0.44 m/s, ◇ = 0.66 m/s, △ = 1.20 m/s, ▽ = 1.57 m/s. Test conditions: D_f = 0.05 g chitosan/g TSS, L_p = 7.1 m, Dose point = P-0

retention time becomes larger, given constant the pipe flow velocities. This is confirmed in Table 7-3.

Flotation trials were conducted at G_{avg} values of 1130-3540 s^{-1}. The effect of G_{avg} on algal rise rates is shown in Table 7-4. The results indicate that the rise rate at given total pipe length depend on G_{avg}. Maximal rise rate with chitosan was observed at G_{avg} of 2160 s^{-1} when the total pipe length was 12.9 m. The effect of $\Sigma G_i \cdot t_{di}$ on rise rates is shown in Figures 7-16 and 7-17. With a constant pipe length, there was a correlation between Vr_2 and Vr_3, and $\Sigma G_i \cdot t_{di}$. Maximal rise rate with alum was observde at $\Sigma G_i \cdot t_{di}$ of 30,000 with total pipe length of 12.9 m, whereas that was at $\Sigma G_i \cdot t_{di}$ of 40,000 with 7.1 m of pipe length.

7.4.4 Effect of flocculation variables

Algal rise rates with alum were significantly affected by the location of the dose point (Fig. 7-18). The highest rise rates were observed when flocculant was added at P-0. Slightly lower rates were obtained when P-1 was the dose point and substantially lower rates were obtained when P-2 was the dose point. At the pipe flow velocity of 1.05 m/s employed in these trials, the corresponding flocculant contact times were 0.05, 0.42 and 6.64 seconds.

The trials represented in Figure 7-18 were conducted at constant Lp and Vp of 12.9 m and 1.05 m/s, respectively. In order to determine whether flocculant contact time exerted a consistent effect, data from all trials with Df = 1.2 g alum/g TSS were plotted in Figure 7-19. Results with alum in this study was in good agreement with those of Arbelaez et al. (1983).

Table 7-3. Effect of total pipe retention time an algal rise rates.

Pipe flow velocity (m/s)	Rise rate of alum flocculated algae (Vr_2) at $\Delta DO = 10\ g/m^3$ [a]					
	Σt_d (s)					
	9.5	10.7	16.1	17.7	25.5	28.5
0.66				29.4 ± 2.5		
0.69					35.9 ± 1.4	
1.09		34.6 ± 1.1	25.2 ± 1.9			13.7 ± 1.3
1.84	21.8 ± 0.7					
	Rise rate of chitosan flocculated algae (Vr_3) at $\Delta DO = 10\ g/m^3$ [b]					
	Σt_d (s)					
	7.5	9.8	14.6	17.7	26.1	26.6	60.1
0.19							36.4 ± 0.9
0.44						95.2 ± 22.5	
0.66				133.0 ± 18.7			
1.20		94.0 ± 10.1	37.0 ± 3.5		28.2 ± 5.1		
1.57	62.6 ± 4.3						

Test conditions

[a] Alum: Df = 12.9 g/g TSS
Dose point = P-0

[b] Chitosan: Df = 0.05 g/g TSS
Dose point = P-0

Table 7-4. Effect of overall mean velocity gradient on algal rise rates.

Total pipe length (m)	Rise rate of alum flocculated algae (Vr_2) to $\Delta DO = 10$ g/m^3 [a] G_{avg} (s^{-1}) 1330	1540	2010	2160	2570	2790
7.1				29.4 ± 2.5		34.6 ± 1.1
12.9		35.9 ± 1.4	25.2 ± 1.9		21.8 ± 0.7	
25.8	13.4 ± 1.3					

Total pipe length (m)	Rise rate of chitosan flocculated algae (Vr_3) to $\Delta DO = 10$ g/m^3 [b] G_{avg} (s^{-1}) 1130	1450	1720	2160	2160	3010	3540
7.1	36.4 ± 0.9		95.2 ± 22.5		133 ± 18.7	94.0 ± 10.1	62.6 ± 4.3
12.9				37.0 ± 3.5			
25.8		28.2 ± 5.1					

Test conditions:

[a] Alum: Df = 1.2 g/g TSS
Dose point = P-0

[b] Chitosan: Df = 0.05 g/g TSS
Dose point = P-0

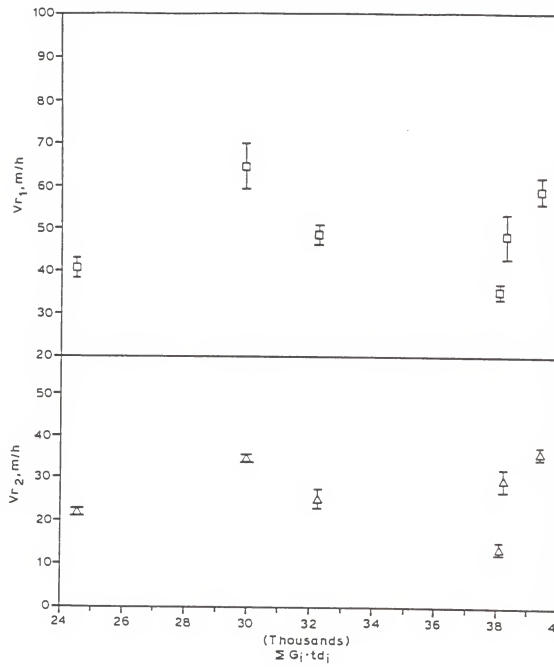

Figure 7-16. Effect of $\Sigma G_i.t_{di}$ on normalized rise rates (ΔDO = 10 g/m^3) of alum flocculated algae. Bars represent the 95% confidence intervals. Test conditions: D_f = 1.2 g/g TSS, Dose point = P-0

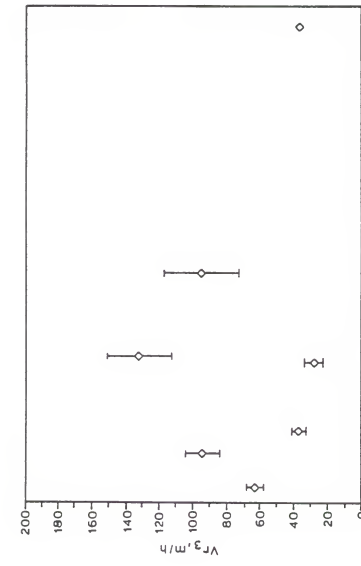

Figure 7-17. Effect of $\Sigma G_i \cdot t_{d_i}$ on normalized rise rates ($\Delta DO = 10\ g/m^3$) of chitosan flocculated algae. Bars represent the 95% confidence intervals. Test conditions: $D_f = 0.05$ g/g TSS, Dose point = P-0

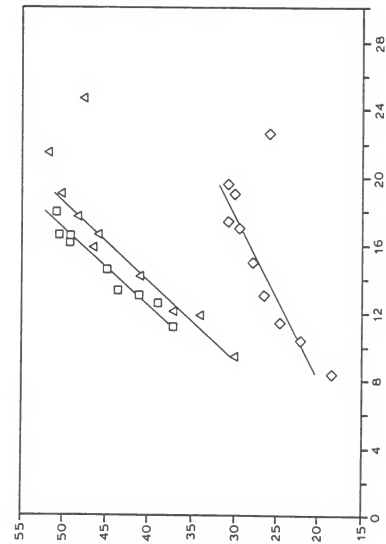

Figure 7-18. Effect of dose point on rise rates of alum flocculated algae. Dose points: □ = P-0, Δ = P-1, ◇ = P-2. Test conditions: D_f = 1.2 g/g TSS, L_p =12.9 m, V_p =1.05 m/s.

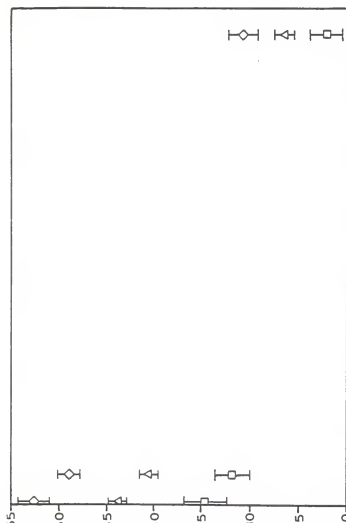

Figure 7-19. Effect of flocculant contact time on normalized rise rates of alum flocculated algae. DO: □ = 10 g/m^3, Δ = 14 g/m^3, ◇ = 18 g/m3. Bars represent 95% confidence intervals. Test condition: D_f = 1.2 g/g TSS, L_p =12.9 m, V_p =1.05 m/s

Algal rise rates with chitosan were insensitive to the location of the dose point (Fig. 7-20). This would indicate that flocculant contact time was not a significant variable. The plot of normalized algal rise rate versus contact time shown in Figure 7-21 corroborates this conclusion.

The effect of mean velocity gradient downstream of the dose point on rise rates of alum flocculated algae is shown in Figure 7-22. The analogous data for chitosan is shown in Figure 7-23.

Figure 7-24 shows the correlation of $G \cdot t_c$ with rise rates of alum flocculated algae. Rise rates of chitosan flocculated algae are correlated with $G \cdot t_c$ in Figure 7-25. At a constant pipe length, rise rates (Vr_2 and Vr_3) depended on $G \cdot t_c$. With chitosan, the correlation between rise rate (Vr_3) and $G \cdot t_c$ was significant. Maximal rise rate (Vr_3) was observed at a $G \cdot t_c$ of about 15.

7.4.5 Effect of algal species

Figure 7-26 shows algae removal efficiency as a function of flocculant dosage for two different cultures. The dosage required to achieve a given removal efficiency with Synechocystis was generally greater than that required with a mixture of Chlorella and Monodus, regardless of which harvesting method was employed. The dosage requirement for 50% removal by sedimentation with Chlorella/Monodus dominated culture was 0.64 g alum/g TSS and 21 mg chitosan/g TSS, respectively. With Synechocystis dominated culture the dosage requirements for 50% removal by sedimentation were 1.03 g alum/g TSS and 37 mg chitosan/g TSS, respectively. This would indicate that flocculant dosage requirements were controlled by the surface area of algal cells. Removal

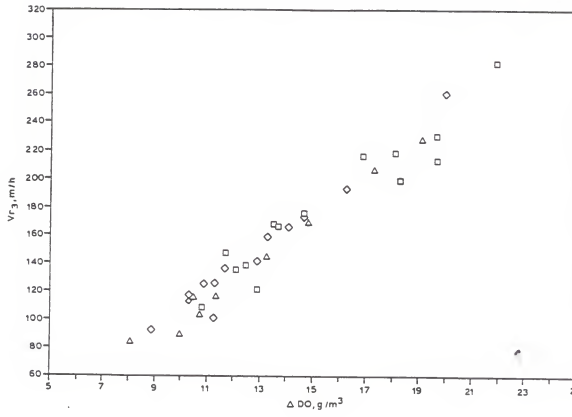

Figure 7-20. Effect of dose point on rise rates of chitosan flocculated algae. Dose points: □ = P-0, △ = P-1, ◇ = P-2. Test conditions: D_f = 0.05 g/g TSS, L_p = 12.9 m, V_p = 0.87 m/s.

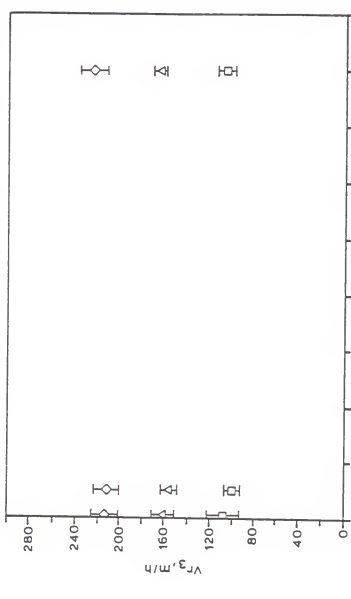

Figure 7-21. Effect of flocculant contact time on normalized rise rates of chitosan flocculated algae. ΔDO: □ = 10 g/m^3, Δ = 14 g/m^3, ◇ = 18 g/m^3. Bars represent the 95% confidence intervals. Test conditions: D_f = 0.05 g/g TSS. L_p = 12.9 m, V_p = 0.87 m/s.

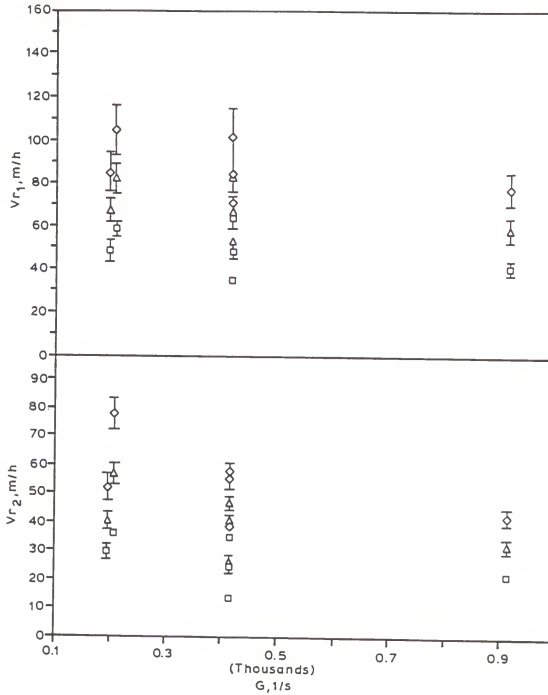

Figure 7-22. Effect of mean velocity gradient downstream of the dose point on normalized rise rates of alum flocculated algae. ΔDO: □ = 10 g/m^3, △ = 14 g/m^3, ◇ = 18 g/m^3. Bars represent the 95% confidence intervals. Test conditions: D_f = 1.2 g/g TSS.

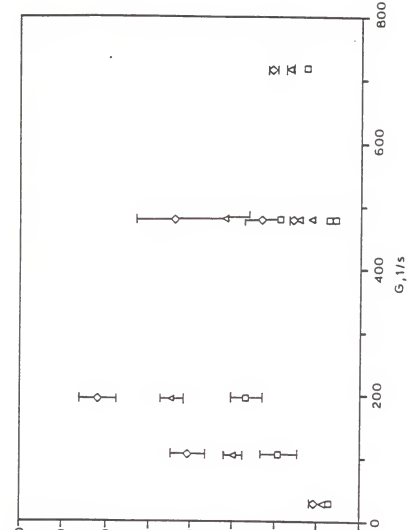

Figure 7-23. Effect of mean velocity gradient downstream of the dose point on normalized rise rates of chitosan flocculated algae. ΔDO: □ = 10 g/m^3, Δ = 14 g/m^3, ◇= 18 g/m^3. Bars represent the 95% confidence intervals. Test conditions: D_E = 0.05 g/g TSS.

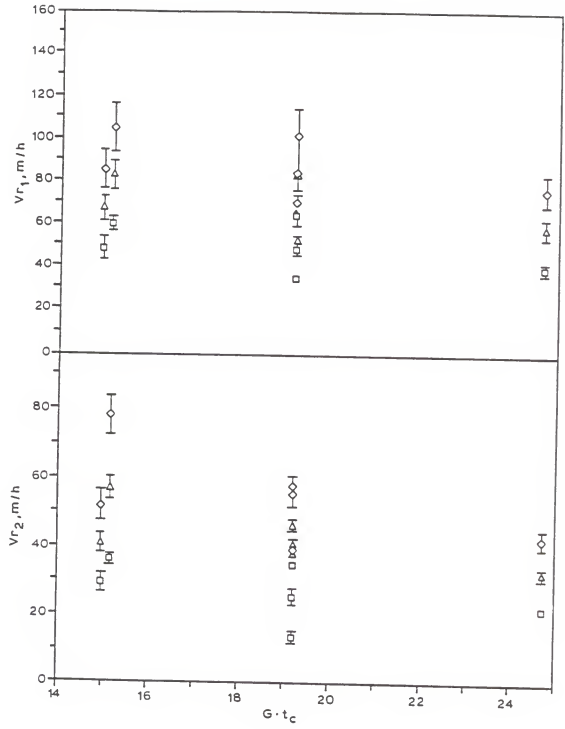

Figure 7-24. Effect of G·t_c on normalized rise rates of alum flocculated algae. ΔDO: □ = 10 g/m^3, △ = 14 g/m^3, ◇ = 18 g/m^3. Bars represent the 95% confidence intervals. Test conditions: D_f = 1.2 g/g TSS.

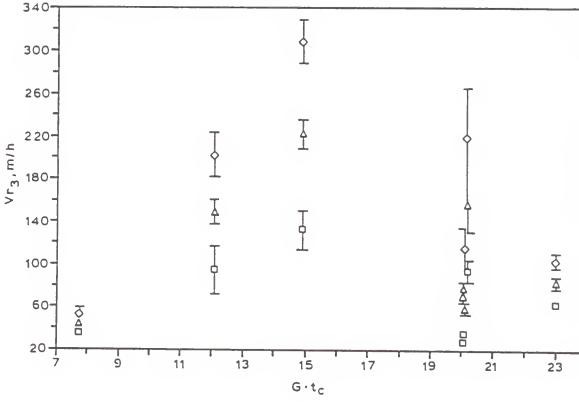

Figure 7-25. Effect of $G \cdot t_c$ on normalized rise rates of chitosan flocculated algae. ΔDO: □ = 10 g/m^3, △ = 14 g/m^3, ◇ = 18 g/m^3. Bars represent the 95% confidence intervals. Test conditions: D_f = 0.05 g/g TSS.

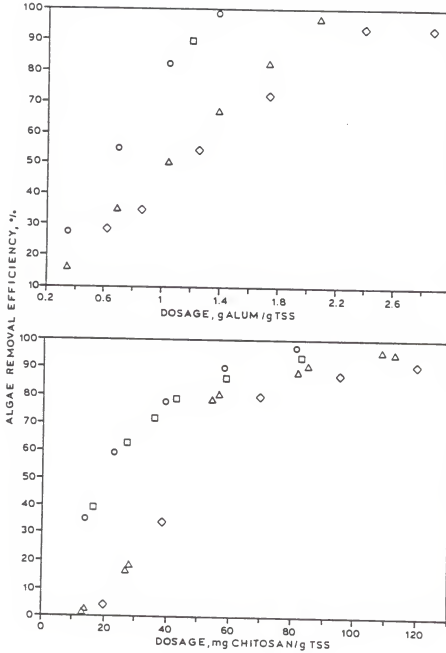

Figure 7-26. Effect of algal species on removal efficiency with alum (top) and chitosan (bottom). □ = Chlorella/Monodus - autoflotation, ○ = Chlorella/Monodus - Jar test, ◇ = Synechocystis - autoflotation, △ = Synechocystis - Jar test.

efficiencies achieved with autoflotaion and sedimentation were similar.

7.4.6 Effect of algal physiological state

When ammonium hydroxide was added to control rotifers, the color of the C-4 culture changed from a dark, grass green to a pale, yellow green within a period of 24 hours. The original color reappeared after 5 days. Figure 7-27 indicates that algal rise rates on successive days of the recovery period were significantly different. The rise rates at one day after the addition of ammonium hydroxide were lowest. Algal rise rates gradually increased to the same magnitude as those observed before chemical treatment.

These results may be due to reduction of the overall mass transfer coefficient (K_La) for oxygen. After addition of ammonium hydroxide to control rotifers that had reached a density of more than 100,000/L, considerable organic matter (rotifer cacasses and polymers from lysed algal cells) was released. This matter could have reduced surface tension. For example, algal polymers acting as surface active agent, and soluble organics released from the decomposing rotifers could have surface active properties. Small amounts of surface active compounds in water were shown to reduce K_La in single bubble system (Mancy and Okun, 1960; McKeown and Okun, 1960; Koide et al., 1976; Gurol and Nekouinaini, 1985) and swarms of bubbles systems (Eckenfelder and Barnhart, 1961).

7.4.7 Characteristics of recoverable algae

Figure 7-28 indicates that with alum as flocculant, the total solids concentration of floated algal slurry was strongly

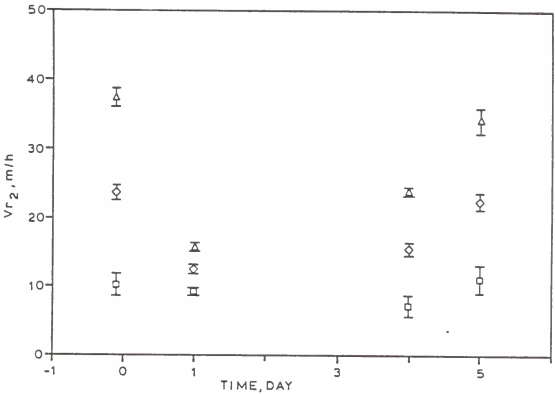

Figure 7-27. Recovery of algal rise rates following ammonium hydroxide addition to algal culture. ΔDO: □ = 6 g/m^3, ◇ = 10 g/m^3, △ = 14 g/m^3. Bars represent the 95% confidence intervals.

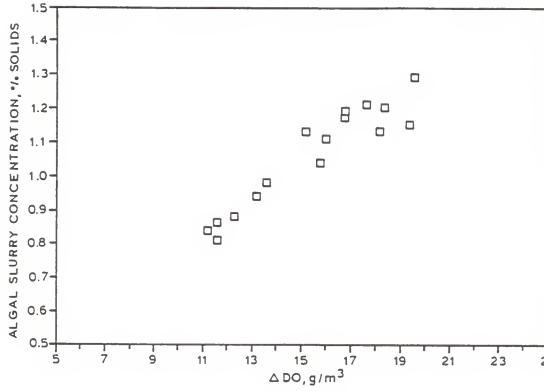

Figure 7-28. Effect of dissolved oxygen excess on the total solids concentration of floated alum-algae slurry. Test conditions: D_f = 1.2 g/g TSS, L_p = 12.9 m, V_p = 0.87 m/s, Dose point = P-0.

dependent on the dissolved oxygen excess. Figure 7-29 shows that the total solids concentration of chitosan-algae slurry was also strongly dependent on dissolved oxygen excess.

Table 7-5 shows that the ash content of alum-algae slurries was much greater than that of chitosan-algae slurries. The chitosan-flocculated algae were only marginally greater in ash content than the algal biomass itself. Total solids concentration of floated slurries exceeded those of settled slurry by a factor in excess of 20.

7.4.8 Mechanisms of alum and chitosan flocculation and their effect on autoflotation

The results obtained in the pilot autoflotation trials showed that rise rates of algal flocs with alum were consistently less than those with chitosan. The maximum rise rate (Vr_2) with alum was observed to be 70 m/h, whereas that with chitosan was approximately 300 m/h. This can be explained by differences between the two flocculants in terms of (1) physicochemical characteristics and (2) floating mechanism.

The chitosan used in these experiments was made from snow crab shells and can be characterized as a high molecular weight biopolymer. Its molecular weight and viscosity are over 1×10^6 grams and 590 cps in 1% solution, respectively. This polymer is strong enough to minimize floc shear in turbulent environments. Alum is an inorganic coagulant which can be easily hydrolyzed to form hydroxide complexes. Hydrolysis increases progressively with the age of Al(III) solution. Eventually, colloidal hydroxo polymers and, ultimately, insoluble hydrous aluminum oxide precipitates are formed. The hydrolysis products, not the

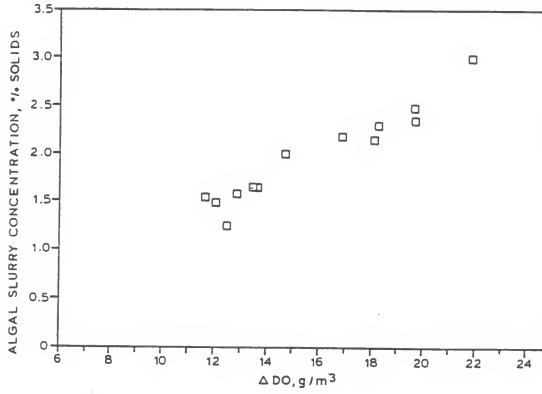

Figure 7-29. Effect of dissolved oxygen excess on the total solids concentration of floated chitosan-algae slurry. Test conditions: D_f = 0.05 g/g TSS, L_p = 12.9 m, V_p = 0.87 m/s.

Table 7-5. Total solids concentration and ash content of algal slurries in relation to flocculant type and harvesting method[a,b]

Flocculant	Harvesting method	No. of samples	TS (g/L))	Ash(% of TS)
chitosan	sedimentation	3	10.4 ± 0.1	16.5 ± 0.17
chitosan	autoflotation	13	20.4 ± 5.0	16.3 ± 0.93
alum	autoflotation	12	11.3 ± 1.0	39.6 ± 1.04

[a]Major algal species = Chlorella sp. + Monodus sp.
[b]Ash content of algal biomass = 14.7 ± 0.56%

aluminum ion, cause particulate destabilization through charge neutralization and bridging (Mattson, 1928; Stumm and O'Melia, 1960).

The rise of algal flocs with alum began 10-60 seconds after switching to the batch mode. An induction period (i.e., lag time) was also observed by Arbelaez et al. (1983). However, the lag times they obtained were on the order of minutes. This could be attributed to the relatively great total pipe length (35.7 m) which they used. In the present experiments, induction time depended on ΔDO concentration in culture medium.

Alum addition at points other than P-0 reduced rise rates. This can be explained as follows. As oxygen-supersaturated culture passed through the pump, valves and plastic pipe, minute gas bubbles, a few microns in size, were produced. These bubbles could become attached and entrapped in algal flocs if the flocs were strong enough to avoid shear in the turbulent pipe environment. Gas bubbles dettached from flocs can coalesce to form larger bubbles, which quickly rise to water surface in the flotation cylinder without attaching to algal flocs. This phenomenon was observed with alum as flocculant. Coarse bubbles were observed just after switching to the batch mode. Then, fine flocs reaggregated and flocs floated to water surface. Fine bubbles were observed in the floated algal slurry. With chitosan, algal flocs rose up instantly. Since chitosan flocs should be stronger than alum flocs, chitosan flocs were able to form and persist in the pipe.

A second factor explaining the difference in performance between the two flocculants is the hydrophobicity of algal flocs. Gochin and Solari (1983) showed that the hydrophobicity of flocs can considerably increase their adherence to air bubbles. Thus, chitosan, which consists of at least 60,000 carbon atoms per molecule, is expected to adhere more tightly to gas bubbles than alum flocs.

Third, there might be a fundamental difference in floating mechanisms between alum and chitosan. With alum, mechanism (2) (i.e., the growth of bubbles from nuclei on solids within floc networks) is evidently the primary mechanism of flotation, because an induction period (lag time) was always observed. Although dissolved oxygen is precipitated in the pipe, supersaturated DO is not fully converted to gas bubbles. DO in the cylinder is still supersaturated. After the induction period, bubbles appear in flocs and grow from nuclei on the solids. With chitosan, floating mechanisms (1) (i.e., the entrapment of pre-formed bubbles within condensing floc structure) and (3) (i.e., the attachment of flocs to bubbles on collision) are likely most important in algal autoflotation. Generated bubbles can be easily entrapped and remain in chitosan flocs.

7.4.9 Application of autoflotaion harvesting algae in field scale

Chitosan was an excellent flocculant for harvesting algae (Chlorella and Monodus) at the field scale. The dosage giving a removal efficiency of 69.7 $\pm$ 4.4 %, as obtained with 5 random samples of effluent, was 15.7 g/m^3 or 26 mg chitosan/g TSS. Comparing this results with those of autoflotation in pilot scale

experiments (Fig. 7-26), the dose requirements were very similar to each other. A dosage of 26 mg chitosan/g TSS with both pilot scale autoflotation experiments and jar test experiments gave 63% removal of algae. Even though DO in surface water was initially 22 g/m^3, problems with insufficient or marginal DO levels occurred after approximately 1 hour of harvesting. ΔDO in the tank was sometimes dropped to 2 g/m^3. Under this condition, portions of the accumulated algal mat sometimes sank and were lost to the subnatant. This expained why the fluctuation of removal efficeincies (max.=74.3%; min.= 65.6 %) in this experiment was so large. After 105 min of harvesting, the thickness of the accumulated algal mat was ranged from 5 to 7 cm. Its solids concentration averaged to 2.2 %.

7.4.10 Application of autoflotation and its primary economic evaluation

Application of autoflotation using alum as a sole means of algae harvesting has been judged to be useful and economical in this UF system throughout most of year. Application of chitosan as primary flocculant is considered to be very promising alternative.

Based on the results of this study, the chemical cost of chitosan can be compared to the cost of conventional flocculant (i.e., alum). If mass productions of chitosan were achieved in the U.S., chitosan product could be estimated in 1976 to sell at best for as little as $1.00 and worst for about $2.50 per pound. From the Japan commercial unit, chitosan was sold in 1976 by $2.12-2.72 per pound (Kohn, 1976). Recently, the manufacturer who supplied chitosan for this study, anounced that the cost of

chitosan was $7.50-8.50 per pound (1985). They also predicted that price of chitosan could drop to less than $4.00 per pound after operation of Louisiana plant (personal communication with Kytek, Inc., 1985). If we consider chitosan price of $4.00 or less, the chemical cost would be at worst $0.38 for harvesting 1 kg of dried algae. Whereas, using alum priced at $200/tonne, the chemical cost would be $0.24 per kg of algal solids. Although the cost of harvesting with chitosan is more expensive than that with alum, if consider the quality of harvested biomass, chitosan flocculation is thought to be promising. It is also evident that if the cost of chitosan could be droped to $2.50 by mass production, its cost would be cheaper than that of alum flocculation, and no doubt be economical. Also, the biomass recovered would be healthy and be free of the substantial ash content imparted by alum precipitation.

CHAPTER 8
SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

8.1 Summary

This research was divided into three main phases. In the first phase, the effect of environmental and management variables on bioflocculation of waste-grown microalgae was studied. In the second, chemical flocculation using chitosan was investigated in order to evaluate it as an alternative to compensate the inconsistent performance of bioflocculation. In the third, autoflotation using chitosan and alum was examined as an algal harvesting process.

In the first phase of this research, two laboratory and three field experiments were carried out. In the laboratory, the first experiment focused on determination of the effect of nutrient composition in algal cultures on bioflocculation. Nutrient composition was manipulated by the use of different pH conditions. The ratio of COD/N/P in each medium was determined, but the differences were not significant. However, the divalent cation (e.g., Ca^{2+} and Mg^{2+}) concentration of each culture differed considerably. In cultures treated at pH 10 and 11, Ca^{2+} and Mg^{2+} was precipitated as $Ca(OH)_2$ and $Mg(OH)_2$. After day 21, considerable amounts of algal flocs were observed. Removal efficiencies in the control, pH 9 treated, pH 10 treated, and pH

11 treated medium were 80, 85, 71 and 46%, respectively. The second experiment conducted in the laboratory aimed to define whether or not the presence of native and externally seeded bacteria have an important role in bioflocculation. Cultures taken from the high-rate pond were sterilized partially or completely by chlorine or hydrogen peroxide. After that, half of the cultures received a small portion of activated sludge. Best removal was observed in the unseeded/unsterilized cultures, followed by the partially sterilized and seeded cultures, and the seeded/sterilized cultures. Completely sterilized cultures were not flocculated. In the three field experiments, partial bioflocculation was achieved. Ash-free biopolymer concentration gradually increased as the extent of bioflocculation increased. Extracted biopolymer from bioflocculated algae could always be characterized as a yellowish, gum-type which contained little ash. When the algae was completely dispersed, a whitish, flour-type biopolymer was extracted which contained 7-12 times more ash than the yellowish. The degree of algal bioflocculation was proportional to the density of yellow color in the extracted biopolymer. The yellow color in the extracted biopolymer might have originated from the algal pigment exuded from dead algae. Most algal bioflocculation and yellowish, gum-type biopolymer was observed when algal growth phase entered the late stationary or declining phase.

The second phase of this research focused on the application of chitosan as a flocculant for algal harvesting. Initially, the chitosan flocculation conditions, such as, optimum pH, mixing

speed and duration, were investigated. Flocculation efficiency was sharply increased as pH was reduced below 7.0 and a maximum was observed between pH 3-4. The flocculation efficiency was also influenced by mixing conditions. Maximum flocculation was observed at the high mixing intensity (G) of 165 s^{-1} and 60 seconds duration, and low mixing intensity of 57 s^{-1} and over 15 minutes duration. As expected, chitosan flocculation was also influenced by algal species and surface area. The dose requirement for the 50% algal removal in the Chlorella and Monodus dominated cultures was about 50% less than for Synechocystis. In field experiments, chitosan dose requirement was observed to be gradually reduced as algal bioflocculation developed. Also, brief mixing of the culture was found to reduce the chitosan dose requirement considerably. Biological and physicochemical parameters were further examined for the reduction of chitosan dose requirement. First, the effect of pre-treatment with an oxidant on chitosan flocculation was examined. Pre-treatment with 5 mg Cl_2/L or 1 mg H_2O_2/L increased removal efficiency up to 10%. In the second study, physiological condition of algae was evaluated. A minimum chitosan dose requirement was observed when algal growth was in the early exponential phase. In this growth phase, biopolymer production (which was characterized as the whitish, flour-type) was at a minimum. As algal growth entered the stationary phase, more chitosan was required for the same degree of algal removal. In the third set of experiments, the effect of mixing on chitosan dosage requirement was examined. Results of these experiments showed that loose biopolymer (of the

whitish, flour-type) considerably hindered chitosan flocculation. The production of this kind of polymer could be reduced by mixing. One or two day's mixing of unmixed cultures reduced the chitosan dose requirement by 30-40% for 50% algal removal. Further mixing did not significantly improve the chitosan flocculation efficiency. Also, elevation of ionic strength of the culture reduced the efficiency of chitosan flocculation. If the effects of extracellular loose biopolymer and ionic strength were prevented, the chitosan dose requirement could be reduced to 10% that of the control.

In the third phase of this research, the performance of the flocculation-autoflotation process with chitosan and alum was compared with that of the flocculation-sedimentation process with respect to harvesting time, slurry density, and algal removal efficiency by pilot scale test. A more rapidly formed and denser slurry was obtained in the flocculation-autoflotation process than in the flocculation-sedimentation process. Chitosan was evaluated as the potential alternative for the primary flocculant in the autoflotation process. Comparing rise rates obtained with chitosan and with alum, the rise rate with chitosan was found to be significantly greater than that with alum. In order to define variables which affect the performance of autoflotation, the effect of dissolved oxygen concentration, applicator configuration, flocculant contact time (i.e., dose point) and physiological condition of culture were examined. After this, field-scale (>50,000 litres) autoflotation was carried out. The result of this experiment was comparable to that of the pilot

scale test. Algae removal efficiency was equal to or better than that of the flocculation-sedimentation process.

8.2 Conclusions

Phase I: Bioflocculation

1. The extent of algal bioflocculation was positively correlated with ash-free biopolymer concentration. Algal bioflocculation was influenced by the total amount of ash-free extracellular biopolymer rather than by its composition.

2. Lowered Ca^{2+} and Mg^{2+} concentration in the medium adversely affected the degree of algal bioflocculation.

3. The extent of algal bioflocculation depended on both the quality and quantity of biopolymer present. The quality of biopolymer was a more important variable in the development of algal bioflocculation.

4. Algal bioflocculation was consistently observed in both laboratory and field when the extracted extracellular biopolymer was of the yellowish, gum-type. Whitish, high-ash type of biopolymer was observed when algae remained in stable suspension.

5. Oxidant treated/non-seeded cultures were inferior to controls with respect to algal bioflocculation. The oxidant treated/seeded cultures were more bioflocculated than the oxidant treated/non-seeded cultures.

6. The presence of bacteria in algal cultures was essential for the development of algal bioflocculation. The native bacteria in algal cultures were more important to bioflocculation than were the seeded ones.

Phase II: Algae flocculation with chitosan

7. Algae flocculation with chitosan was influenced by pH and physical conditions of flocculation. The flocculation efficiency of chitosan increased with duration of fast and slow mixing and at slow mixing speed.

8. Pre-treatment with oxidant improved the flocculation efficiency with chitosan.

9. Chitosan dose requirement was strongly influenced by algal surface area and dominant algal species.

10. Bioflocculation reduced the chitosan dose requirement. Initial development of small flocs by bioflocculation before harvesting by chitosan proved to be one of the more promising strategies for reducing the chitosan dose requirement.

11. Flocculation efficiency with chitosan was dependent on the type and quantity of extracted biopolymer. Chiotsan flocculation was impaired when the algae was associated with a whitish, powdery type biopolymer, whereas it was promoted when the algae contained the yellow, gum-type biopolymer.

12. Mixing of the culture reduced the chitosan dose requirement by the reducing the production of loose biopolymer. The intensity of mixing of cultures was more important in the cultures dominated by Chlorella and Monodus than in those dominated by Synechocystis.

13. Algal flocculation with chitosan was affected by algal growth phase.

14. The ionic strength of the culture medium and the presence of loose polymer significantly affected the flocculation with chitosan.

Phase III: Autoflotation

15. The rise rate of algal slurry during autoflotation was strongly correlated with the amount of dissolved oxygen in excess of normal saturation. Higher rise rate was generally observed when the excess dissolved oxygen was greater.

16. Higher excess dissolved oxygen reduced the flocculation period when alum was used, and caused the production of denser floating algal slurries.

17. Rise rates of algal slurries depended on the type of flocculant used. Rise rates with chitosan were about 5 times greater than those with alum.

18. Mean velocity gradient, retention time, and flocculant contact time, which depended on configuation of the autoflotation vessel, affected rise rates of algal slurries.

19. Rise rate with alum depended on flocculant dose point, whereas rise rate with chitosan did not. The highest rise rate was observed when alum was injected 5 cm below the bottom of flotation cylinder.

20. There was no difference of algal removal efficiency between flocculation-sedimentation and flocculation-autoflotation. As for reduction of harvesting time and production of denser algal slurries, autoflotation was much superior to sedimentation.

21. The rise rate of algal slurries was influenced by the change of physiochemical and biological characteristics of culture.

8.3. Recommendations

1. The effect of an individual nutrient component (e.g., C, N, P, and S) on the production of algae extracellular biopolymer must be defined. Also, the effect of interaction between biopolymer and divalent cations (e.g., Ca^{2+} and Mg^{2+}) on algal bioflocculation should be further evaluated.

2. The effect of interactions between floc-forming bacteria and algae on bioflocculation should be studied. Likewise, the effect of mutual relationship between floc-forming bacteria, non-floc-forming bacterial populations (i.e., algal lytic bacteria) and algae needs to be examined.

3. Specific chemical components in the yellowish, gum-type extracellular biopolymer should be determined as flocculation factor. Furthermore, its origin and production process should be defined.

4. The effect of interaction between the concentration of the chitosan solution and mean velocity gradient in the flocculation tank needs to be evaluated. Also, settled volume and its concentration should be examined as a function of mixing intensity of initial rapid mixing.

5. The injection of hydrogen peroxide should be evaluated both as means of pre-disinfection and as a supply of excess oxygen when the amount of dissolved oxygen is not sufficient for autoflotation.

APPENDIX A

EVALUATION OF MICRO JAR TEST PROCEDURE

APPENDIX A
DEVELOPMENT OF MICRO-JAR TEST PROCEDURE

A.1 Introduction

The jar test procedure is widely used to evaluate flocculants and aid in process control. Sample volumes required in the tests described by Black et al. (1957), Lai et al. (1975), and Cornwell and Bishop (1983) require at least one litre of sample. The limited algal culture volumes which can be produced in the laboratory prevent use of these procedures. The purpose of this study was to evaluate a micro-jar test procedure utilizing smaller sample sizes.

A.2 Theoretical considerations relating to the determination of mean velocity gradient in jar tests

Camp and Stein (1943) applied Stokes' theory to relate the total mixing energy input introduced to a basin of water to what they termed the root-mean-square velocity gradient (G):

$$G = (W/\mu)^{1/2} \qquad \text{(A-1)}$$

where

W = dissipation function = power loss per unit volume of fluid (W/m^3),

μ = absolute viscosity of the fluid ($N \cdot s/m^2$).

The value of W depends on the geometry of the stators, rotors, and containers and on the speed of the rotors. Accurate values of W can be determined by measurement of the torque input to the liquid at various speeds and temperatures:

$$W = (2\pi sT)/V \qquad \text{(A-2)}$$

where

s = rotor speed (rev/s),

T = torque input (N·m), and

V = liquid volume (m^3).

Camp (1968) reported that the floc size and volume concentration may be varied over a wide ranges by changing values of G. The value G, then, was shown to be an important parameter in coagulation and flocculation processes.

Lai et al. (1975) demonstrated that, although flow patterns were different, impellers of different shapes produced the same G values as long as their projected areas were the same. It was observed that variation of the distance between the impellers and the vessel bottom in unbaffled jars did not change the energy input. They also reported that the installation of baffles increased the energy input; however, all fully baffled jars had the same energy input regardless of baffle size or geometry with a given size, shape and rotational speed of the impeller.

A.3 Materials and methods

A.3.1 Standard jar test apparatus and procedure

The standard jar test apparatus (Phipps and Bird) has paddle dimensions of 2.5 cm height and 3.8 cm length (Fig. A-1), where length is measured radially outward from the center of the shaft. One litre aliquots of sample were transfered to acrylic plastic having dimensions of 11.5 x 11.5 x 20 cm. Sample pH was adjusted to 6.5 if the flocculant was chitosan. Flash mixing (125 rev/min, $G = 165\ s^{-1}$) was initiated immediately after addition of flocculant. After one min, mixing was reduced to 20 rev/min ($G = 13\ s^{-1}$) and held at this level for 15 min. Finally, a quiescent settling period of 30 min was allowed. At the end of the settling period, 10 mL of supernatant was taken from each jar at 2 cm below the surface and analyzed for optical density. Removal efficiency was calculated as described in section 2.3.4.

A.3.2 Micro jar test procedure

The micro jar test apparatus employed the Phipps and Bird motor drive system with smaller paddles. The paddles were made from thin (1 mm) plastic. The size and projected area of the paddles are shown in Table A-1. Jars were made of acrylic plastic and had dimensions of 5.0 x 5.0 x 10.0 cm (Fig. A-2). The sample size was 100 mL.

A.3.3 Measurement of mean velocity gradient in micro jar test apparatus

Torque was calculated according to the following equation:

$$T = \Delta F \times R \qquad \text{(A-3)}$$

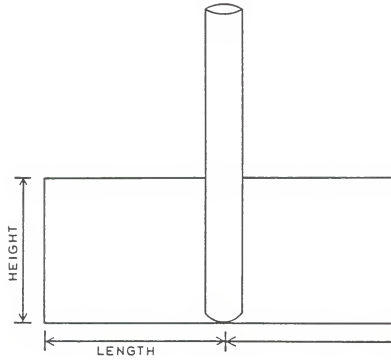

Figure A-1. Configuration of paddle.

Table A-1. Size and projected area of paddles used in micro jar test procedure.

No.	Size (cm) (length x height)	Projected area (cm^2)
1	1.75 x 1.5	5.25
2	1.75 x 2.0	7.00
3	2.00 x 1.5	6.00

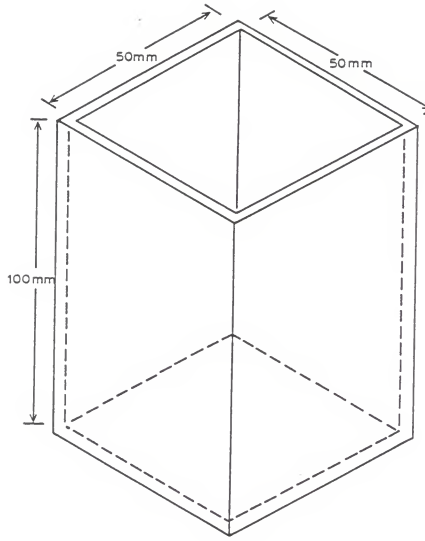

Figure A-2. Micro gator jar.

where ΔF = force measured in the experimental unit (N) and R = length of lever arm (m).

The torque measurements were performed using the test setup depicted in Figure A-3, which was modified from that of Lai et al. (1975). The acrylic jar hung by two 0.1 cm cotton threads attached to a steel bar (dia. = 0.5 cm). This steel bar was suspended by a 0.2 cm cotton thread attached to another steel bar (dia. = 2 cm). Two long cotton threads (dia. = 0.05 cm) were attached to the jar at points A and B from a pulley (point C) and another thread (point D), respectively. At C, a pulley (dia. = 1.0 cm) was installed and one end of the thread was attached to a pan which was loaded with glass beads (0.1 g each). A thread that formed a 115° angle to thread BD was attached at point D at one end and a stand on the other. Rotational speed was measured by a tachometer.

During each measurement the angle BDH was adjusted to 115 $\pm$ 1 degrees by a fine adjusting screw H on the stand. A sharp needle slightly supported the center of the jar bottom (point E) in order to keep the jar from vibrating. The difference between the two weights on the torsion balance was measured and, then divided by tangent 65° for the calculation of the force component of the torque resulting from rotation of the paddle. The torque arm R was the distance between E and J (2.75 cm). The temperature of the water was determined at the time of each torque measurement.

The initial weight differential on torsion balance without paddle rotation was kept constant at 45 g by adjusting the weights of the glass beads at C and weights on the balance (i.e., F = G +

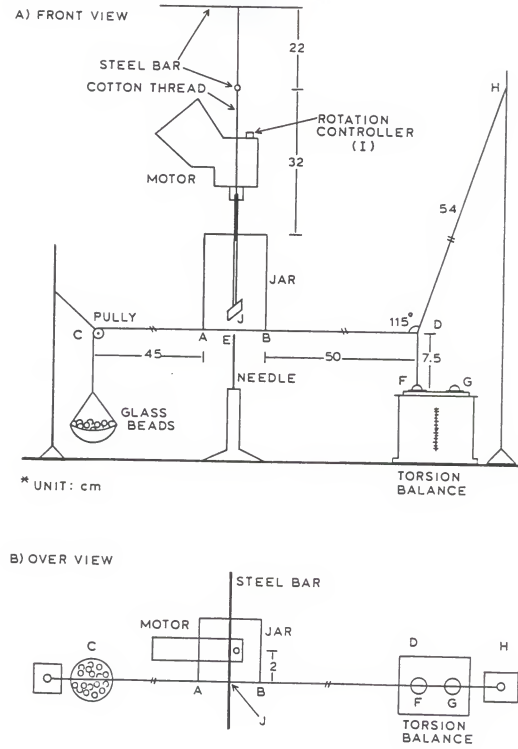

Figure A-3. Set up for torque measurement.

45 g). With paddle rotating clockwise, it was necessary to add glass beads to pan F in order to maintain balancing between F and G. The change in the weight differential (ΔF) was checked by measuring weight of added glass beads.

With ΔF and R known, the torque was calculated and used, in turn, to determine W. From the known values of the viscosity of water at the temperature of the experiment and the values of W, G values were calculated.

A.3.4 Experimental procedure

In the first experiment (L-3A), algal culture was obtained from the field system (C-4). Dominant genera were Chlorella and Monodus. Micro jar test procedure was as follows. Flash mixing immediately after addition of flocculant. After 1 min flash mixing, mixing intensity was reduced to 20 rev/min and maintained for 15 min, followed by 30 min settling. At the end of the settling period, 5 mL of supernatant was removed from each jar at 2 cm below the surface and analyzed for optical density, Algae removal efficiency was calculated as described in section 2.3.4.

In a second experiment (L-3B), algal culture dominated by Chlorella and Monodus (70% of biovolume) and Synechosystis (30% of biovolume) was obtained from the field system (C-4). Rapid mixing (125 rev/min) times of 10, 30, 60 and 90 seconds, followed by slow mixing speeds of 10, 20, 30, 40, 50, and 60 rev/min for 15 min, following one minute of rapid mixing at 125 rev/min, were tested. Supernatant was taken for measuring removal efficiency after 30 min settling.

In both experiments, the pH of samples for chitosan flocculation were adjusted to pH 6.5 using 8 N H_2SO_4. Alum ($Al_2(SO_4)_3 \cdot 18H_2O$) solution was made at a strength of 5%. Chitosan used for the experiments was made from crab shells (Protan lot # 123-121-03). The stock solution was prepared at 1.0% strength in dilute acetic acid as described in section 4.3.2, then diluted to 0.1% with distilled water.

A.4 Results and discussion

The effect of paddle length, paddle height and rotational speed on mean velocity gradient in the micro jar test is shown in Figures A-4 and A-5. The curves paralleled each other and had slopes of approximately 3/2 in the test range. This is in good agreement with Lai et al. (1975). Differences among G values obtained with different paddle lengths were greater than the differences among G values obtained with different paddle heights. With paddle length constant, G values were exactly in proportion to the projected area of the paddles.

Figure A-6 (top) shows that there was little difference in results obtained with the micro and standard jar test procedures when chitosan was the flocculant. Micro jar test results obtained using paddle size of 1.5 x 1.75 cm (height x length) were very close to those of the standard procedure. When alum was used as flocculant (Fig. A-6, bottom), removal efficiencies with the micro jar test procedure was inferior to that obtained with the standard procedure at dosages exceeding 0.5 g alum/g TSS. The results of standard jar and micro jar test with different rapid mixing duration were shown in Figure A-7, top. The effect of slow mixing

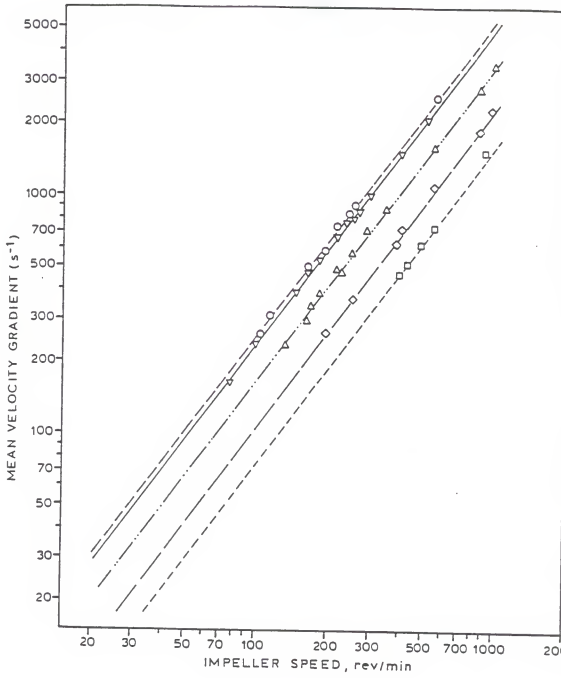

Figure A-4. Effect of paddle length and rotational speed on mean velocity gradient in micro jar test. Sample volume and paddle size (length x height): ○ = 100 mL and 1.75 x 1.5 cm, ▽ = 200 mL and 1.75 x 1.5 cm, △ = 200 mL and 1.5 x 1.5 cm, ◇ = 200 mL 1.25 x 1.5 cm, □ = 200 mL and 1.0 x 1.5 cm.

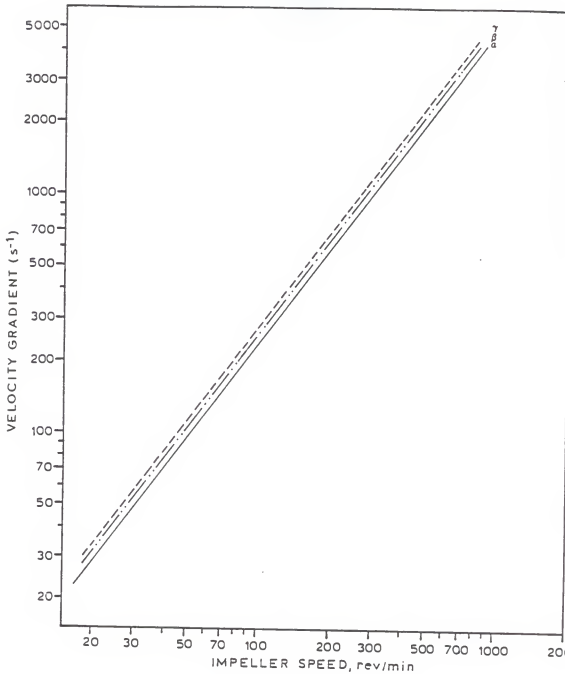

Figure A-5. Effect of paddle height and rotational speed on mean velocity gradient in micro jar test. Paddle size: α = 1.75 x 1.5 cm, β = 1.75 x 2.0 cm, γ = 1.75 x 2.5 cm.

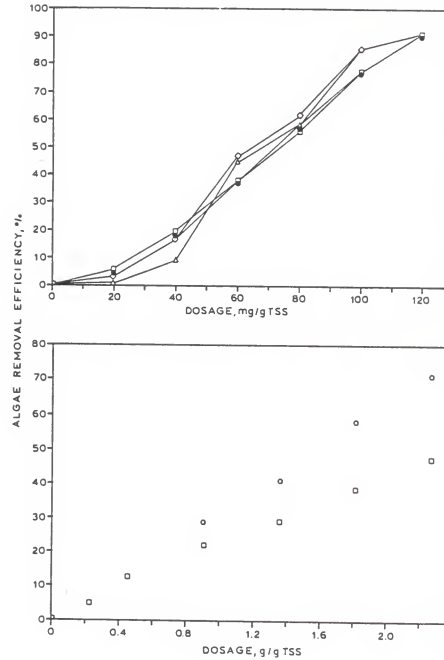

Figure A-6. Comparison of micro and macro jar tests. Top with chitosan as flocculant; bottom - with alum as flocculant. Top □ = macro and paddle size of 3.8 x 2.5 cm, ● = micro and 1.75 x 1.5 cm, ◇ = micro and 1.75 x 2.0 cm, △ = micro and 2.0 x 1.5 cm; bottom: ○ = macro and 3.8 x 2.5 cm, □ = micro and 1.75 x 1.5 cm.

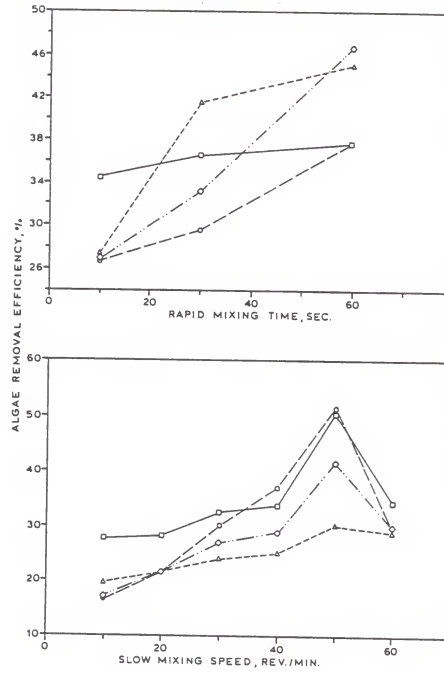

Figure A-7. Top - effect of rapid mixing time on micro and macro jar test results; Bottom - effect of show mixing speed on micro and macro jar test results. Jar type and paddle size: □ = macro and 3.8 x 1.5 cm, ○ = micro and 1.75 x 1.5 cm, ◇ = micro and 1.75 x 2.0 cm, △ = micro and 2.0 x 1.5 cm.

speed between them was shown in Figure A-7, bottom. At 60 second of rapid mixing, the performances of standard jar test and micro jar test using a paddle of 1.5 x 1.75 cm was very similar with chitosan as flocculant. Flocculation curves developed at different slow mixing speeds were also slimiar between standard jar test and micro jar test using a paddle size of 1.5 x 1.75 cm. This indicates that the performance of micro jar test procedure with a paddle size of 1.5 x 1.75 cm was empirically proven to be similar to that of standard jar test procedure if the flocculant was chitosan.

APPENDIX B

EXPERIENCE WITH ALUMINIUM SULFATE AS FLOCCULANT

APPENDIX B
EXPERIENCE WITH ALUMINIUM SULFATE AS FLOCCULANTS

B.1 Mechanism of flocculation

When aluminum sulfate is added to water, it dissociates yielding trivalent Al^{+3} ions which hydrate to form the aquametal complex $Al(H_2O)_6^{+3}$. This complex passes through a series of hydrolytic reactions in which H_2O molecules in the hydration shell are replaced by OH^- ions. This gives rise to the formation of a variety of soluble species, including mononuclear species such as $Al(OH)^{+2}$ and $Al(OH)_2^+$ and polynuclear species such as $Al_8(OH)_{20}^{+4}$. Despite the fact that some of these products have only one or two positive charges, they are quite effective as flocculants because they adsorb strongly onto the surface of most negative colloids. Hydrolysis increases progressively with the age of Al (III) solutions. This can convert positive aluminum complexes into negative ones (Stumm and O'Melia, 1968). Flocculation effects of aged solutions and fresh solutions are quite different (Fair et al., 1968).

According to Stumm and O'Melia (1968), aluminum (III) acomplishes destabilization by two mechanisms: (1) adsorption and charge neutralization and (2) enmeshment in sweep floc. If an aluminum (III) salt is added to water in concentrations less than the solubility limit of the metal hydroxide, the hydrolysis

products will form and adsorb onto particulates, causing destabilization by charge neutralization. When the amount of aluminum (III) added to water exceeds the solubility of the metal hydroxide, the hydrolysis products will appear as kinetic intermediates in the formation of the metal hydroxide precipitate. In this situation charge neutralization and enmeshment in the precipitate both contribute to flocculation.

The charge on hydrolysis products and the precipitation of metal hydroxides are both controlled by pH. Hydrolysis products possess a positive charge at pH values below the iso-electric point of the metal hydroxide. These positively charged species can cause destabilization of negatively charged colloids by adsorption and charge neutralization. Negatively charged species ($Al(OH)_4^-$), which are predominant above the iso-electric point cannot effectively destabilize negatively charged colloids.

Precipitation of amorphous metal hydroxide is necessary for sweep-floc coagulation. The solubility of $AL(OH)_{3(s)}$ is minimal at a particular pH and increases as the pH deviates from that value.

B.2 Alum flocculation of microalgae

Golueke and Oswald (1965) showed that best removal of algae was obtained at pH 6.5. Slightly less removal obtained when pH approached 6.0 or 6.8. Removal efficiency deteriorated significantly when the pH was raised above 7.0. These investigators also found that a mixing time of three minutes was ample at a jar tester's blade-tip velocity of 30.5 cm/s and a settling time as brief as 15 min was sufficient for algae removal.

Shindala and Stewart (1971) found that the optimum dosage was 75 to 100 mg/L of alum (TSS was not given). When this dosage was used, the phosphate removal was 90% and COD removal was 70%.

McGary (1970) reported the results of a complete factorially designed experiment using a jar test apparatus. Tests were performed to determine the economic feasibility of using polyelectrolytes as primary coagulants alone or in combination with alum. Alum was found to be effective for coagulation of algae from high rate oxidation pond effluents. The polyelectrolytes did not reduce the overall cost of algae removal. The minimum cost per unit algal removal was obtained with alum alone (75 to 100 mg/L; TSS was not given).

Moraine et al. (1980) pointed out that the soluble PO_4 concentration influenced the optimal alum dose. The required dose of alum was given by:

$$Al^{+3} = (PO_4^{-3})_s + k\ (TSS) \qquad (B\text{-}1)$$

where

Al^{+3} = alum dose (mM),

$(PO_4^{-3})_s$ = soluble phosphate (mM),

TSS = total suspended solids concentration (g/L), and

k = specific alum dose (mmole Al^{+3}/g TSS).

The coefficient k should be function of effluent characteristics, but was not correlated with such parameters as alkalinity, NH_3, or BOD. It was weakly correlated with temperature and algal type.

Shelef et al. (1984) found that alum dosages for over 90%

removal increased as the ionic strength of the medium increased. They pointed out that high salts concentrations inhibit the flocculation process by reducing the chemical activity of the flocculant and by masking its active functional sites.

In autoflotation tests, van Vuuren and van Duuren (1965) achieved 80% removal of algal solids with an alum dosage of 245-330 mg/L at algal densities of 25-40 mg SS/L (3.2-9.9 g alum/g SS) with culture media pH averaging 10. In further studies using maturation pond effluent, Cillie et al. (1966) reported that alum dosages could be halved by adjusting the initial pH to 6.5 with CO_2. Van Vuuren et al. (1965) applied autoflotation to facultative pond effluent. Typical results indicated a reduction of algal solids from 280 mg/L to 84 mg/L (70% removal) at an alum dosage of 780 mg/L (2.8 g alum/g SS). In 1972, autoflotation was evaluated at a tertiary pond system receiving both municipal and canning wastes (Anon., 1972; Parker et al., 1973). The alum dosage in these trials ranged from 75 to 200 mg/L, depending on a variable SS concentration of 20-160 mg/L (i.e., 1.2-3.8 g alum/g SS). Koopman and Lincoln (1983) reported that with alum dosage of 1.42 g alum/g VSS, a high efficiency of algal separation was achieved with effluents of a high rate pond treating swine waste. They observed that culture media pH was generally in the range of 7.8-9.0, dropping to 6.0-6.5 with alum addition. Addition of HCl during harvests to achieve a pH of 5.5 had no discernible effect. Arbelaez et al. (1983) reported that a dosage of approximately 1.2 g alum/g TSS was sufficient to achieve 90% algal removal by autoflotation.

APPENDIX C

MICELLANEOUS TABLES AND FIGURES

Table C-1. Calculation of G_{avg} for autoflotation trials with alum. Df = 1.2 g/g TSS, dose point = P-0.

Experiment No.	A-1	A-2	A-3	A-4	A-5	A-6
Reactor length (m)	7.1	7.1	12.9	12.9	12.9	25.8
Flow rate (L/s)	1.34	2.21	1.39	2.21	3.73	2.21
Velocity (m/s)	0.66	1.09	0.69	1.09	1.84	1.09
Pipe:						
G_1 (s^{-1})	197	418	208	418	916	418
G_1t_{d1}	2118	2720	3920	4941	6420	9880
Pump:						
G_2 (s^{-1})	6040	7424	6142	7446	8006	7510
G_2t_{d2}	29524	22004	28940	22069	14059	22257
Gate valve:						
G_3 (s^{-1})	31448	36227	31956	36227	36168	36227
G_3t_{d3}	5632	3934	5518	3934	2327	3934
Elbow (90°):						
G_4 (s^{-1})	507	1075	537	1075	2358	1075
G_4t_{d4}	545	700	556	700	910	1402
Elbow (45°):						
G_5 (s^{-1})	570	1207	602	1207	2647	1207
G_5t_{d5}	476	612	485	612	795	612
$\Sigma G_i t_{di}$	38295	29970	39419	32256	24511	38088
Σt_{di} (s)	17.73	10.74	25.52	16.06	9.52	28.55
G_{avg} (s^{-1})	2160	2790	1545	2008	2575	1334

* 3-way valve was considered as 90° elbow

Table C-2. Calculation of G_{avg} for autoflotation trials with chitosan. Df = 0.05 g/g TSS, dose point = P-0.

Experiment No.	C-1	C-2	C-3	C-4	C-5	C-6	C-7
Reactor length (m)	7.1	7.1	7.1	7.1	7.1	12.9	25.8
Flow rate (L/s)	0.38	0.89	1.34	2.43	3.18	2.43	2.43
Velocity (m/s)	0.19	0.44	0.66	1.20	1.57	1.20	1.20
Pipe:							
G_1 (s^{-1})	29	106	196	480	718	478	478
G_1t_{d1}	1115	1718	2110	2841	3250	5140	10324
Pump:							
G_2 (s^{-1})	3243	4940	6036	7963	8928	7878	7970
G_2t_{d2}	55954	36353	29505	21464	18389	21271	21520
Gate valve:							
G_3 (s^{-1})	16953	25774	31448	38902	43141	38358	38358
G_3t_{d3}	10707	6950	5632	3842	3256	3788	3788
Elbow (90°):							
G_4 (s^{-1})	76	274	507	1240	1856	1235	1235
G_4t_{d4}	288	444	545	735	841	732	1464
Elbow (45°):							
G_5 (s^{-1})	173	308	570	1932	2084	1086	1086
G_5t_{d5}	73	388	477	642	734	649	649
$\Sigma G_i t_{di}$	68137	45853	38268	29524	26470	31580	37745
Σt_{di} (s)	60.06	26.64	17.73	9.80	7.47	14.64	26.09
G_{avg} (s^{-1})	1134	1721	2159	3013	3544	2157	1447

* 3-way valve was considered as 90° elbow

Table C-3. Linear regressions of algal rise rate on ΔDO

Flocculant	Rise rate parameter	Exp. No.	$\Sigma G_i \cdot t_{di}$	No. of trials	Y = ax + b a	b	r
Alum	Vr1	A-1	38290	11	4.617	1.780	0.9521
		A-2	29970	14	4.678	17.545	0.9109
		A-3	39420	10	5.741	1.213	0.9660
		A-4	32260	13	4.504	3.049	0.9776
		A-5	24510	10	4.588	-5.104	0.9771
		A-6	38090	13	4.450	-9.401	0.9890
	Vr2	A-1	38290	11	2.799	1.374	0.9698
		A-2	29970	17	2.879	5.825	0.9836
		A-3	39420	10	5.236	-16.460	0.9908
		A-4	32260	15	3.766	-12.442	0.9767
		A-5	24510	10	2.540	-3.594	0.9930
		A-6	38090	13	3.131	-17.859	0.9872
Chitosan	Vr_3	C-1	68140	10	2.118	15.228	0.9543
		C-2	45850	8	13.424	-39.003	0.9498
		C-3	38290	12	21.986	-87.087	0.9815
		C-4	29520	10	15.593	-61.935	0.9332
		C-5	26470	14	5.170	10.860	0.9791
		C-6	31580	9	5.197	-15.019	0.9858
		C-7	37740	11	10.979	-81.631	0.9502

y = rise rate, m/h
x = ΔDO, g/m^3

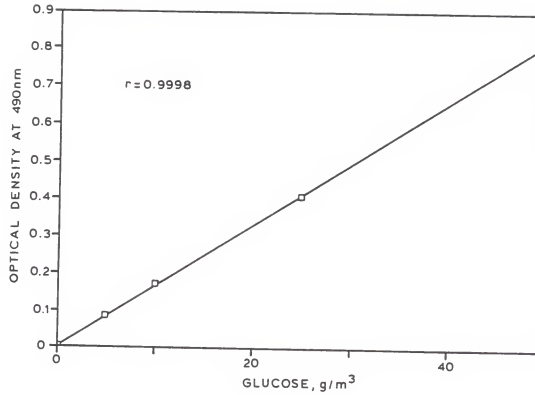

Figure C-1. Standard curve for glucose determination.

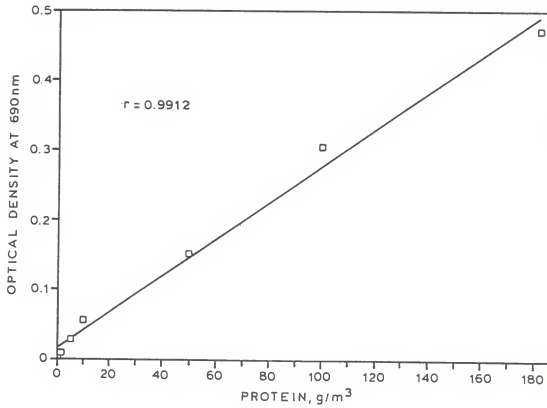

Figure C-2. Standard curve for protein determination.

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BIOGRAPHICAL SKETCH

Sang-ill Lee, son of In-hak and Jeung-ahn, was born March 18, 1953, in the suburbs of Chung-ju, Korea. In March, 1972, he entered Seoul National University, Seoul, Korea, and received the degree of Bachelor of Science (Oceanography) in February 1976. After graduating, he served for three years in the army and then worked for two years for the Union Steel Manufacturing Company as a researcher. He entered the University of Florida in August 1981 and was awarded a Master of Science degree in environmental engineering sciences in December 1984, majoring in water supply and water pollution control. His thesis was entitled "Denitrification with Wastewater Organics". He married Ryang-kyun, daughter of Byung-ho and Young-soon Ko, in August 1980. He has twin boys.

He is a member of Tau Beta Pi, the Korean Scientists and Engineers Association in America, the American Water Works Association, and the Water Pollution Control Federation.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Ben Koopman, Chairman
Associate Professor of Environmental Engineering Sciences

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Edward P. Lincoln, Co-chairman
Associate Professor of Agricultural Engineering

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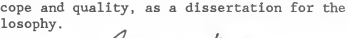

Gabriel Bitton
Professor of Environmental Engineering Sciences

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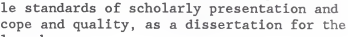

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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This dissertation was submitted to the Graduate Faculty of the College of Engineering and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

May 1987

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